PB81-244477

Acephate, Aldicarb, Carbophenothion, DEF, EPN, Ethoprop, Methyl Parathion, and Phorate; Their Acute and Chronic Toxicity, Bioconcentration Potential, and Persistence as Related to Marine Environments

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

May 81

U.S. Department of Commerce
National Technical Information Service
NTIS

PB81-244477

United States
Environmental Protection
Agency

EPA-600/4-81-041
May 1981

EPA

Research and Development

Acephate, Aldicarb, Carbophenothion, DEF, EPN, Ethoprop, Methyl Parathion, and Phorate: Their Acute and Chronic Toxicity, Bioconcentration Potential, and Persistence as Related to Marine Environments

Prepared for

Office of Pesticides
and Toxic Substances

Prepared by

Environmental Research
Laboratory
Gulf Breeze FL 32561

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/4-81-041	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE Acephate, Aldicarb, Carbophenothion, DEF, EPN, Ethoprop, Methyl Parathion, and Phorate: Their Acute and Chronic Toxicity, Bioconcentration Potential, and Persistence as Related to Marine Environments		5. REPORT DATE May 1981 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Experimental Environments Branch Environmental Research Laboratory, Gulf Breeze		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS		10. PROGRAM ELEMENT NO. A87E1A 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Gulf Breeze Environmental Research Laboratory Office of Research and Development Environmental Protection Agency Gulf Breeze, Florida 32561		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/4

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The toxicity, bioconcentration, and persistence of the pesticides acephate, aldicarb, carbophenothion, DEF, EPN, ethoprop, methyl parathion, and phorate were determined for estuarine environments. Static acute toxicity tests were conducted to determine the 96-h EC50 values for algae, 48-h EC50 values for oyster larvae, and 96-h LC50 values for at least two crustacean and fish species. Flow-through acute toxicity tests, based on measured concentrations, were conducted to determine the 96-h LC50 values of the pesticides for at least two crustacean and fish species. In addition, Maximum Acceptable Toxicant Concentrations (MATC) were determined in life-cycle toxicity tests with mysid shrimp (Mysidopsis bahia) and sheepshead minnows (Cyprinodon variegatus), or in partial life-cycle tests with grass shrimp (Palaemonetes pugio). MATCs were estimated from embryo-juvenile toxicity tests with sheepshead minnows. Persistence studies on carbophenothion, DEF, EPN, methyl parathion, and phorate investigated processes in marine systems that contribute to those pesticides' disappearance. The relative importance of biological and nonbiological processes (including biodegradation, photolysis, hydrolysis, sediment/water partitioning, and volatility) were examined. Bioconcentration factors for fish or mollusks exposed to carbophenothion, EPN, ethoprop, and phorate were determined at steady state or after ≥28-day exposures.

plus or minus

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Aquatic toxicology Pesticides	Acephate, Aldicarb, Carbophenothion, DEF, EPN, Ethoprop, Methyl parathion, and Phorate	06/F

18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/4-81-041
May 1981

ACEPHATE, ALDICARB, CARBOPHENOTHION, DEF, EPN, ETHOPROP, METHYL PARATHION, AND PHORATE: THEIR ACUTE AND CHRONIC TOXICITY, BIOCONCENTRATION POTENTIAL, AND PERSISTENCE AS RELATED TO MARINE ENVIRONMENTS

by

Experimental Environments Branch
Environmental Research Laboratory
U.S. Environmental Protection Agency
Gulf Breeze, Florida 32561

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE, FLORIDA 32561

DISCLAIMER

This report has been reviewed by the Gulf Breeze, Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commerical products constitute endorsement or recommendation for use.

FOREWORD

Protection of our nation's estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of such chemicals into the environment be formulated on a sound scientific basis. As a research laboratory of the U.S. Environmental Protection Agency (EPA), the Environmental Research Laboratory, Gulf Breeze (ERL,GB) conducts toxicological studies of the effects and persistence of pesticides and other chemicals on a single species and on communities of marine animals and plants.

During the last 20 years, this laboratory has developed a defined testing protocol for determining the toxicity and persistence of a particular chemical to marine organisms. A request from EPA's Office of Pesticides and Toxic Substances (OPTS) for the evaluation of eight pesticides gave our scientific staff the opportunity to utilize each bioassay procedure of our testing protocol, namely: (1) acute static toxicity tests; (2) acute flow-through toxicity tests; (3) embryo-juvenile tests; (4) life-cycle tests; (5) benthic community tests; (6) bioconcentration tests; and (7) persistence tests.

Our report on these investigations provides a reference document on the toxicity, bioconcentration potential, and persistence of the eight subject pesticides: acephate, aldicarb, carbophenothion, DEF, EPN, ethoprop, methyl parathion, and phorate. Further, it demonstrates the marine toxicological and analytical capabilities of the Experimental Environments Branch.

Jack I. Lowe

Jack I. Lowe
Chief
Experimental Environments Branch

ABSTRACT

The toxicity, bioconcentration, and persistence of the pesticides acephate, aldicarb, carbophenothion, DEF, EPN, ethoprop, methyl parathion, and phorate were determined for estuarine environments. Static acute toxicity tests were conducted to determine the 96-h EC50 values for algae, 48-h EC50 values for oyster larvae, and 96-h LC50 values for at least two crustacean and fish species. Flow-through acute toxicity tests, based on measured concentrations, were conducted to determine the 96-h LC50 values of the pesticides for at least two crustacean and fish species. In addition, Maximum Acceptable Toxicant Concentrations (MATC) were determined in life-cycle toxicity tests with mysid shrimp (*Mysidopsis bahia*) and sheepshead minnows (*Cyprinodon variegatus*), or in partial life-cycle tests with grass shrimp (*Palaemonetes pugio*). MATCs were estimated from embryo-juvenile toxicity tests with sheepshead minnows. Persistence studies on carbophenothion, DEF, EPN, methyl parathion, and phorate investigated processes in marine systems that contribute to those pesticides' disappearance. The relative importance of biological and nonbiological processes (including biodegradation, photolysis, hydrolysis, sediment/water partitioning, and volatility) were examined. Bioconcentration factors for fish or mollusks exposed to carbophenothion, EPN, ethoprop, and phorate were determined at steady state or after $\geq$28-day exposures.

Acute and chronic toxicities of the pesticides differed markedly. The crustaceans (*Penaeus duorarum* or *M. bahia*) were the most acutely sensitive of the 7 to 10 species tested; frequently, the alga (*Skeletonema costatum*) or the eastern oyster (*Crassostrea virginica*) were least sensitive. The lowest 96-h LC50 in µg/ℓ for each pesticide was: acephate, 3,800; aldicarb, 12; carbophenothion, 0.47; DEF, 4.6; EPN, 0.29; ethoprop, 6.3; methyl parathion, 0.77; and phorate, 0.11. Of the 14 chronic toxicity tests, *P. pugio* was the most sensitive to carbophenothion, and *M. bahia* was the most sensitive species for the other pesticides. The MATC in µg/ℓ for the species most sensitive to each pesticide was: acephate, 588 to 1,400; aldicarb, 1.0 to 1.5; carbophenothion, 0.22 to 0.36; DEF, <0.34; EPN, 0.44 to 3.4; ethoprop, 0.36 to 0.62; methyl parathion, 0.11 to 0.34; and phorate, 0.09 to 0.21.

Persistence and bioconcentration tests provide further insight needed in hazard assessment. Bioconcentration factors were less than 100 in organisms exposed to ethoprop and phorate, and less than 10,000 for carbophenothion and EPN. Persistence studies in sediment/water systems that contained natural populations of microorganisms demonstrated the following half-lives (in days): 0.5, phorate; 1.0, methyl parathion; 2.2, DEF; 2.3, EPN; and 6.5, carbophenothion. Persistence of these chemicals was most frequently diminished by biological processes in water or sediment, or both.

Evaluation of the relative hazards of chemicals to aquatic environments requires that information on toxicity, accumulation potential, and expected environmental concentrations be compared. Data on acute and chronic toxicity and accumulation potentials of these eight pesticides and on their persistence will be useful for hazard assessment. Additionally, methods used will be useful for future generation of data to assess other chemicals.

CONTENTS

Foreword....................iii
Abstract.................... iv
Figures....................
Tables....................
Acknowledgement....................

1. Introduction.................... 1
2. Materials and Methods.................... 3
    Test organisms.................... 3
    Test pesticides.................... 3
    Test water.................... 3
        Environmental Research Laboratory, Gulf Breeze (ERL,GB)...... 3
        Bionomics Marine Research Laboratory (BMRL).................... 4
    Acute toxicity tests.................... 4
        Static algal tests.................... 4
        Static oyster larval tests.................... 5
        Static copepod test.................... 6
        Static invertebrate and fish tests.................... 6
        Flow-through macrocrustacean and fish tests.................... 6
    Chronic toxicity tests.................... 8
        Mysid shrimp entire life-cycle test.................... 8
            Acephate test.................... 8
            Aldicarb test.................... 9
            Ethoprop test.................... 9
            EPN, phorate, carbophenothion, and DEF tests.................... 10
            Methyl parathion test.................... 10
        Grass shrimp partial life-cycle toxicity test.................... 11
        Sheepshead minnow embryo/juvenile tests.................... 12
            Aldicarb and ethoprop tests.................... 13
            Carbophenothion and phorate tests (ERL,GB).................... 14
        Sheepshead minnow entire life-cycle test.................... 16
        Benthic animal community test.................... 17
        Bioconcentration tests.................... 18
            Carbophenothion.................... 18
            EPN.................... 19
        Persistence.................... 19
            Log P.................... 19
            Persistence test.................... 19
        Pesticide analytical methods.................... 20
            Water.................... 21
            Tissue.................... 21
            Phorate and carbophenothion.................... 21
            EPN and DEF.................... 21

Methyl parathion 21
Sediment 22
Analytical equipment and quality assurance 22
Instrumentation 22
Quality control 22
Acetylcholinesterase (AChE) 22
Statistical methods 23
Algal test 23
Oyster embryo test 23
Acute toxicity test 23
Mysid shrimp entire life-cycle tests 23
Acephate, aldicarb, and ethoprop 23
Carbophenothion, DEF, EPN, methyl parathion and phorate 23
Grass shrimp partial life-cycle tests 24
Carbophenothion 24
Sheepshead minnow embryo/juvenile and entire life-cycle test 24
Bioconcentration tests 25
Persistence tests and Log P 25
3. Results and Discussion 26
Acephate 26
Results 26
Acute static toxicity tests 26
Algae 26
Invertebrates 26
Fishes 26
Acute flow-through toxicity tests 26
Invertebrates 26
Fishes 26
Mysid shrimp entire life-cycle test 26
Discussion 27
Aldicarb 28
Results 28
Acute static toxicity tests 28
Algae 28
Invertebrates 28
Fishes 29
Acute flow-through toxicity test 29
Invertebrates 29
Fishes 29
Mysid shrimp entire life-cycle test 29
Sheepshead minnow embryo/juvenile test 29
Discussion 30
Carbophenothion 31
Results 31
Acute static toxicity tests 31
Algae 31
Invertebrates 31
Fishes 31
Acute flow-through toxicity tests 31
Invertebrates 31
Fishes 31

Mysid shrimp entire life-cycle test.......................... 32
Grass shrimp entire life-cycle test.......................... 32
Sheepshead minnow embryo/juvenile test....................... 34
Benthic animal community test................................ 35
Bioconcentration... 35
Persistence.. 36
Discussion... 36
DEF.. 37
Results.. 37
Acute static toxicity tests.................................. 37
Algae.. 37
Invertebrates.. 37
Fishes... 37
Acute flow-through toxicity tests............................ 38
Invertebrates.. 38
Fishes... 38
Mysid shrimp entire life-cycle test.......................... 38
Persistence.. 38
Discussion... 39
EPN.. 40
Results.. 40
Acute static toxicity tests.................................. 40
Algae.. 40
Invertebrates.. 40
Fishes... 41
Acute flow-through toxicity tests............................ 41
Invertebrates.. 41
Fishes... 41
Mysid shrimp entire life-cycle tests......................... 41
Sheepshead minnow entire life-cycle tests.................... 41
Survival and signs of poisoning in parental fish............. 41
Growth of parental fish...................................... 42
Reproduction... 42
Survival, growth, and signs of poisoning in progeny....... 43
MATC and application factor.................................. 44
Bioconcentration... 44
Acetylcholinesterase activity................................ 44
Bioconcentration tests....................................... 45
Persistence.. 45
Discussion... 46
Ethoprop... 47
Results.. 47
Acute static toxicity tests.................................. 47
Algae.. 47
Invertebrates.. 47
Fishes... 47
Acute flow-through toxicity tests............................ 47
Invertebrates.. 47
Fishes... 47
Mysid shrimp entire life-cycle test.......................... 48
Sheepshead minnow embryo/juvenile test....................... 48
Discussion... 48

Methyl parathion.................. 49
Results.. 49
Acute static toxicity tests.................................. 49
Algae.. 49
Invertebrates.. 49
Fishes... 50
Acute flow-through toxicity tests........................... 50
Invertebrates.. 50
Fishes... 50
Mysid shrimp entire life-cycle test......................... 50
Persistence... 51
Discussion... 51
Phorate.. 53
Results.. 53
Acute static toxicity tests.................................. 53
Algae.. 53
Invertebrates.. 53
Fishes... 53
Acute flow-through toxicity tests........................... 53
Invertebrates.. 53
Fishes... 53
Mysid shrimp entire life-cycle test......................... 54
Sheepshead minnow embryo/juvenile tests..................... 54
Persistence... 55
Discussion... 55

References.. 57

Appendices.. 126

A-1. Description of acephate used in tests.......................... 126
A-2. Description of aldicarb used in tests.......................... 127
A-3. Description of carbophenothion used in tests................... 128
A-4. Description of DEF used in tests............................... 129
A-5. Description of ethoprop used in tests.......................... 130
A-6. Description of EPN used in tests............................... 131
A-7. Description of methyl parathion used in tests.................. 132
A-8. Description of phorate used in tests........................... 133
B. Chemical methods for the analyses of acephate (Orthene^{R}), aldicarb (Temik^{R}), carbophenothion (Trithion^{R}), ethoprop (Mocap^{R}) and methyl parathion in seawater and tissue samples... 135
C-1. Acute (96-h) toxicity of eight pesticides to the marine diatom (*Skeletonema costatum*) in static tests.................. 158
C-2a. Number of normal eastern oyster (*Crassostrea virginica*) embryos per milliliter counted following 48 h of exposure to acephate to static, unaerated seawater........................ 162
C-2b. Toxicity of acephate to embryos of eastern oysters (*Crassostrea virginica*) exposed for 48 h in static, unaerated seawater...... 163
C-3. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to acephate in static acute toxicity tests using 20 animals per concentration............................ 164

C-4. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to acephate in static acute toxicity test using 20 fish per concentration.................. 165
C-5. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to acephate in static acute toxicity tests using 30 animals per concentration...................................... 166
C-6. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to acephate in flowing, natural seawater of 29±1 °/oo salinity and 22±1°C.................................... 167
C-7. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to acephate in flowing, natural seawater of 28-30 °/oo salinity and 22±1 C.............................. 168
C-8. Ranges of dissolved oxygen and pH measured in seawater from acute static and flow-through toxicity tests........................ 169
C-9. Test concentrations and mortality of pinfish (*Lagodon rhomboides*) exposed to acephate in flowing, natural seawater of 28-30 °/oo salinity and 22±1 C.. 171
C-10. Test concentrations and percentage of sheepshead minnows (*Cyprinodon variegatus*) exhibiting complete loss of equilibrium and lack of escape response after exposure to acephate in flowing, natural seawater.................................... 172
D-1a. Number of normal eastern oyster (*Crassostrea virginica*) embryos per milliliter counted following 48 h of exposure to aldicarb in static, unaerated seawater................................. 173
D-1b. Toxicity of aldicarb to embryos of eastern oyster (*Crassostrea virginica*) exposed for 48 h in static, unaerated seawater...... 174
D-2. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to aldicarb in static acute toxicity tests using natural seawater adjusted to 20 °/oo; temperature, 26±1°C... 175
D-3. Test concentrations and mortality of postlarval shrimp (*Penaeus stylirostris*) exposed to aldicarb in static acute toxicity tests... 176
D-4. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to aldicarb in flowing natural seawater.. 177
D-5. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to aldicarb in static acute toxicity tests using ten
D-6. Test concentration and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to aldicarb in a flowing seawater test......... 179
D-7. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to aldicarb in a flowing seawater test..... 180
D-8. Test concentration and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to aldicarb in a flowing seawater test... 181
D-9. Test concentrations and mortaltiy of pinfish (*Lagodon rhomboides*) exposed to aldicarb in a flowing seawater test... 182
E-1a. Number of normal eastern oyster (*Crassostrea virginica*) embryos per milliliter counted following 48-h exposure to carbophenothion (Trithion^{R}) in static, unaerated seawater............ 183

E-1b. Toxicity of carbophenothion to embryos of eastern oysters (Crassostrea virginica) exposed for 48 h in static, unaerated seawater........ 184
E-2. Test concentrations and mortality of mysid shrimp (Mysidopsis bahia) exposed to carbophenothion in static acute toxicity tests using 20 animals per concentration........ 185
E-3. Test concentrations and mortality of postlarval penaeid shrimp (Penaeus stylirostris) exposed to carbophenothion in static acute toxicity test using 20 animals per concentration....... 186
E-4. Test concentrations and mortality of sheepshead minnows (Cyprinodon variegatus) exposed to carbophenothion in static acute toxicity tests using 20 animals per concentration...... 187
E-5. Test concentrations and mortality of spot (Leiostomus xanthurus) exposed to carbophenothion in static acute toxicity tests using 20 animals per concentration........ 188
E-6. Test concentrations and mortality of mysid shrimp (Mysidopsis bahia) exposed to carbophenothion in a flowing seawater test. 189
E-7. Test concentrations and mortality of pink shrimp (Penaeus duorarum) exposed to carbophenothion in a flowing seawater test........ 190
E-8. Test concentrations and mortality of grass shrimp (Palaemonetes pugio) larvae (1- to 7-day old) exposed to carbophenothion in a flowing seawater test........ 191
E-9. Test concentrations and mortality of grass shrimp (Palaemonetes pugio) exposed to carbophenothion in a flowing seawater test. 192
E-10. Test concentrations and mortality of wild-stock grass shrimp (Palaemonetes pugio) exposed to carbophenothion in a flowing seawater test........ 193
E-11. Test concentrations and mortality of sheepshead minnows (Cyprinodon variegatus) exposed to carbophenothion in a flowing seawater test........ 194
E-12. Test concentrations and mortality of Atlantic silversides (Menidia menidia) exposed to carbophenothion in a flowing seawater test........ 195
E-13. Test concentrations and mortality of spot (Leiostomus xanthurus) exposed to carbophenothion in a flowing seawater test....... 196
E-14. Test concentrations and mortality of pinfish (Lagodon rhomboides) exposed to carbophenothion in a flowing seawater test........ 197
E-15. Mean salinity and dissolved oxygen during a 249-day partial life-cycle toxicity test of carbophenothion with grass shrimp (Palaemonetes pugio)........ 198
F-1a. Number of normal eastern oyster (Crassostrea virginica) embryos per milliliter counted following 48 h of exposure to DEF in static, unaerated seawater........ 199
F-1b. Toxicity of DEF to embryos of eastern oysters (Crassostrea virginica) exposed for 48 h in static, unaerated seawater... 200
F-2. Test concentrations and mortality of mysid shrimp (Mysidopsis bahia) exposed to DEF in static acute toxicity tests using 20 animals per concentration........ 201

F-3. Test concentrations and mortality of postlarval shrimp (*Penaeus stylirostris*) exposed to DEF in static acute toxicity tests using 20 animals per concentration.......................... 202
F-4. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to DEF in static acute toxicity tests using 20 animals per concentration........... 203
F-5. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to DEF in static acute toxicity tests using 20 animals per concentration.......................... 204
F-6. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to DEF in a flowing seawater test............ 205
F-7. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to DEF in a flowing seawater test......... 206
F-8. Test concentration and mortality of wild-stock grass shrimp (*Palaemonetes pugio*) exposed to DEF in flowing seawater tests 207
F-9. Test concentrations and mortality of pinfish (*Lagodon rhomboides*) exposed to DEF in flowing seawater test......... 208
F-10. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to DEF in flowing seawater tests..................... 209
F-11. Test concentrations and mortality of sheepshead minnow (*Cyprinodon variegatus*) exposed to DEF in a flowing seawater test.. 210
G-1a. Number of normal eastern oyster (*Crassostrea virginica*) embryo per milliliter counted following 48 h of exposure to EPN in static, unaerated seawater................................. 211
G-1b. Toxicity of EPN to embryos of eastern oysters (*Crassostrea virginica*) exposed for 48 hours in static, unaerated seawater 212
G-2. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to EPN in static acute toxicity tests using 20 animals per concentration................................ 213
G-3. Test concentrations and mortality of postlarval penaeid shrimp (*Penaeus stylirostris*) exposed to EPN in static acute toxicit tests using 40 or 60 animals per concentration............... 214
H-6. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to ethoprop in a flowing seawater test......... 232
H-7. Test concentration and mortality of pink shrimp (*Penaeus duorarum*) exposed to ethoprop in a flowing seawater test...... 233
H-8. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to ethoprop in a flowing seawater test.. 234
H-9. Test concentrations and mortality of pinfish (*Lagodon rhomboides*) exposed to ethoprop in a flowing seawater test.... 235
I-1a. Number of normal eastern oyster (*Crassostrea virginica*) embryos per milliliter counted following 48 h of exposure to methyl parathion in static unaerated seawater........................ 236
I-1b. Toxicity of methyl parathion to embryos of eastern oysters (*Crassostrea virginica*) exposed for 48 h in static, unaerated seawater.. 237
I-2. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to methyl parathion in static acute toxicity tests using 20 animals per concentration..................... 238

I-3. Test concentrations and mortality of postlarval penaeid shrimp (*Penaeus stylirostris*) exposed to methyl parathion in static acute toxicity test using 20 animals per concentration........ 239

I-4. Test concentrations and mortality of calanoid copepod (*Acartia tonsa*) exposed for 96 h to methyl parathion in static, unaerated seawater.. 240

I-5. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to methyl parathion in static acute toxicity tests using 20 animals per concentration....... 241

I-6. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to methyl parathion in static acute toxicity tests using 20 animals per concentration........................... 242

I-7. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to methyl parathion in a flowing seawater test. 243

I-8. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to methyl parathion in a flowing seawater tests.. 244

I-9. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to methyl parathion in flowing, natural seawater...... 245

J-1a. Number of normal eastern oyster (*Crassostrea virginica*) embryos per milliliter counted following 48 h of exposure to phorate in static, unaerated seawater................................. 246

J-1b. Toxicity of phorate to embryos of eastern oysters (*Crassostrea virginica*) exposed for 48 h in static, unaerated seawater..... 247

J-2. Test concentrations and mortality of postlarval penaeid shrimp (*Penaeus stylirostris*) exposed to phorate in static acute toxicity tests using 20 animals per concentration............. 248

J-3. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to phorate in static acute toxicity tests using 20 animals per concentration.................................. 249

J-7. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to phorate in a flowing seawater test.......... 253

J-8. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to phorate in flowing seawater test.. 254

J-9. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to phorate in flowing seawater test.................. 255

FIGURES

Number Page

1. Effects of carbophenothion on spawning response of grass shrimp (Palaemonetes pugio) in a partial life-cycle toxicity test....... 63
2. A. longitudinal, horizontal section of a normal sheepshead minnow from control group. B. similar section from fish exposed to 2.8 μg carbophenothion per liter from the zygote stage for 28 days....... 64
3. Uptake and depuration of carbophenothion in whole-body tissues of spot (Leiostomus xanthurus) in a flowing seawater bioassay....... 65
4. Uptake and depuration of EPN in whole-body tissues of pin fish (Lagodon rhomboides) in a flowing seawater bioassay... 66
5. The effect of measured concentrations of phorate on mortality of sheepshead minnows (Cyprinodon variegatus) in a 28-day embryo/juvenile toxicity test at 30°C....... 67

TABLES

Number Page

1-a. Scientists or laboratory responsible for conducting acute static toxicity tests 68
1-b. Scientist or laboratory responsible for conducting acute flow-through toxicity tests 69
1-c. Scientist or laboratory responsible for designated tests and sections of this document 70
2. Composition of mixes added to algal growth media 71
3. Profile of sediment used in persistence studies 72
4-a. Acute toxicity of acephate, aldicarb, carbophenothion, and DEF to estuarine organisms in static tests 73
4-b. Acute toxicity of EPN, ethoprop, methyl parathion, and phorate to estuarine organisms in static tests 74
5-a. Acute toxicity of acephate, aldicarb, carbophenothion, and DEF to estuarine organisms in flowing seawater tests 75
5-b. Acute toxicity of EPN, ethoprop, methyl parathion, and phorate to organisms in flowing seawater tests 76
6. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to acephate 77
7. Number of offspring produced per female of mysid shrimp (*Mysidopsis bahia*) exposed to acephate in a chronic (28-day) exposure in natural, flowing seawater 78
8. Results of chronic toxicity tests with acephate, aldicarb, carbophenothion, DEF, EPN, ethoprop, methyl parathion, and phorate 79
9. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to aldicarb 80
10. Number of offspring produced per female of mysid shrimp (*Mysidopsis bahia*) exposed to aldicarb in a chronic (28-day) exposure in natural, flowing seawater 81
11. Survival of embryo and juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of aldicarb in seawater at 29±1°C 82
12. Standard lengths and survival of juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of aldicarb for 28 days 83
13. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to carbophenothion 84
14. Number of offspring per female of mysid shrimp (*Mysidopsis bahia*) exposed to carbophenothion in a chronic (28-day) exposure in natural, flowing seawater 85

Number Page

15. Survival of F_1 generation of mysids (*Mysidopsis bahia*) continuously exposed to carbophenothion.......................... 86
16. Growth (head-tail length) of parental generation mysid shrimp (*Mysidopsis bahia*) exposed to carbophenothion in a chronic (28-day) exposure in natural, flowing seawater................ 87
17. Survival of adult grass shrimp (*Palaemonetes pugio*) continuously exposed to carbophenothion for 249 days in a partial life-cycle test.. 88
18. Effect of carbophenothion of fecundity of grass shrimp (*Palaemonetes pugio*).. 89
19. Survival of F_1 generation grass shrimp (*Palaemonetes pugio*) larvae reared under conditions of continued exposure to carbophenothion... 90
20. Effect of carbophenothion on weight (g) of grass shrimp (*Palaemonetes pugio*) exposed in a partial life-cycle test..... 91
21. Concentrations of carbophenothion (μg/g) whole body, wet weight in parental generation grass shrimp (*Palaemonetes pugio*), and bioconcentration factors (concentration measured in tissue divided by the average concentration in exposure water) at termination of a 249-day partial life-cycle toxicity test.. 92
22. Survival of embryonic and juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of carbophenothion in seawater at 30°C......................... 93
23. Standard length, tissue concentrations, and bioconcentration factors (concentration measured in whole body, wet weight divided by average measured water concentration) for juvenile sheepshead minnows (*Cyprinodon variegatus*) continuously exposed to various concentrations of carbophenothion in seawater at 30°C.. 94
24. Animals collected from control aquaria and aquaria exposed to carbophenothion in eight-week benthic community study......... 95
25. Total number of individuals and species (in parentheses), by phylum, collected in an eight-week benthic community test with carbophenothion.. 97
26. Octanol/water partition coefficients (Log P) of methyl parathion, phorate, EPN, carbophenothion, and DEF, as determined by reversed phase high pressure liquid chromatography... ... 98
27. Results of persistence studies with carbophenothion............ 99
28. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to DEF......... 100
29. Number of offspring per female mysid shrimp (*Mysidopsis bahia*) exposed to DEF in a 28-day life-cycle toxicity test.... 101
30. Results of persistence studies with DEF....................... 102
31. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to EPN.............. 103
32. Number of offspring per female mysid shrimp (*Mysidopsis bahia*) exposed to EPN in a chronic (28-day) exposure in natural flowing seawater...................................... 104

Number | Page

33. Percentage survival of sheepshead minnows (*Cyprinodon variegatus*) continuously exposed to EPN for 265 days in an entire life-cycle toxicity test 105
34. Effect of EPN on average standard length (mm) of sheepshead minnows (*Cyprinodon variegatus*) exposed for an entire life-cycle 106
35. Egg production by sheepshead minnows (*Cyprinodon variegatus*) continuously exposed to measured concentrations of EPN in flowing seawater at 30°C 107
36. Average dissolved oxygen concentrations (mg/ℓ) during a 265-day toxicity test in which sheepshead minnows (*Cyprinodon variegatus*) were continuously exposed to measured concentrations of EPN at 30°C 108
37. Survival of embryos, fry, and juvenile sheepshead minnows (*Cyprinodon variegatus*) and standard lengths of surviving fish continuously exposed to measured concentrations of EPN for 28 days 109
38. Concentrations of EPN (µg/g, whole body, wet weight) measured in selected life stages of the sheepshead minnow (*Cyprinodon variegatus*) in a 265-day life-cycle toxicity test 110
39. Bioconcentration factors (concentration measured in tissue divided by the average concentration measured in exposure water) for EPN in selected life-stages of the sheepshead minnow (*Cyprinodon variegatus*) in a 265-day life-cycle toxicity test .. 111
40. Acetylcholinesterase activity, expressed as micromoles of acetylcholine hydrolyzed/hour/mg brain tissue x 100, in brains from sheepshead minnows (*Cyprinodon variegatus*) that were continuously exposed to EPN 112
41. Results of persistence studies with EPN 113
42. Mortality (percentage killed) of mysids (*Mysidopsis bahia*) during a chronic (28-day) exposure to ethoprop 114
43. Number of offspring produced per female of mysid shrimp (*Mysidopsis bahia*) exposed to ethoprop in a chronic (28-day) exposure in natural, flowing seawater 115
44. Survival of embryo and juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of ethoprop in seawater at 30±1°C 116
45. Standard lengths, tissue concentrations, and bioconcentration factors of ethoprop in juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of ethoprop for 28 days 117
46. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (24-day) exposure to methyl parathion 118
47. Number of offspring per female mysid shrimp (*Mysidopsis bahia*) exposed to methyl parathion in a chronic (24-day) exposure in natural, flowing seawater 119
48. Results of persistence studies with methyl parthion 120
49. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to phorate 121
50. Number of offspring per female mysid shrimp (*Mysidopsis bahia*) exposed to phorate in a chronic (28-day) exposure in natural flowing seawater 122

Number Page

51. Survival of embryonic and juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of phorate in seawater for 28 days at 30°C.................... 123

52. Standard lengths, tissue concentrations, and bioconcentration factors of phorate in juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of phorate for 28 days.. 124

53. Results of persistence studies with phorate.................... 125

ACKNOWLEDGMENT

Material in this report was prepared by the Experimental Environments Branch of the Environmental Research Laboratory, Gulf Breeze (ERL,GB) and a contractor, Bionomics Marine Research Laboratory, EG&G International, Inc., Pensacola, Florida. Scientists and laboratories responsible for designated tests are identified in Tables 1-a, 1-b, and 1-c (p. 68-70). The contribution of many individuals who assisted in the preparation of the report is appreciated. Special thanks is extended to Dr. Nelson Cooley, editorial advisor; Betty L. Jackson, technical editor; and Dorothy Delk, typist. Dr. Jerry L. Oglesby performed statistical analyses on the benthic community test, bioconcentration tests, and chronic toxicity tests conducted at ERL,GB.

INTRODUCTION

Use of the pesticides acephate, EPN, methyl parathion, phorate, aldicarb (Temik^R), carbophenothion (Trithion^R), DEF, and ethoprop (Mocap^R) has increased, especially in the southern states, because of cancellations in the registration or restrictions in the labeling of certain chlorinated insecticides and herbicides. (Mention of commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.) Due to this increased usage, the Office of Pesticide Programs (OPP) of the U.S. Environmental Protection Agency (EPA) requested that the Agency's Environmental Research Laboratory, Gulf Breeze (ERL,GB), Florida conduct laboratory experiments to determine the toxicity and persistence of these pesticides in the marine environment. Tests were conducted either at ERL,GB or under contract at EG&G Bionomics Marine Research Laboratory (BMRL), Pensacola, Florida.

This report includes the results of testing with the above eight pesticides. Acute static and flow-through toxicity tests were conducted initially on the alga (*Skeletonema costatum*), oyster larvae (*Crassostrea virginica*), mysid shrimp (*Mysidopsis bahia*), penaeid shrimp (*Penaeus duorarum* or *P. stylirostris*), grass shrimp (*Palaemonetes pugio*), copepod (*Acartia tonsa*), Atlantic silversides (*Menidia menidia*), pinfish (*Lagodon rhomboides*), sheepshead minnows (*Cyprinodon variegatus*), and spot (*Leiostomus xanthurus*). The species tested were selected on the basis of seasonal availability, ease of culturing, and sensitivity to pesticides. Chronic toxicity tests included mysid shrimp and sheepshead minnow entire life-cycle, grass shrimp partial life-cycle, and sheepshead minnow embryo/juvenile tests. Specific tests were selected on the basis of relative acute sensitivities of fish and invertebrates and persistence of the pesticide. Generally, if the LC50s differed by more than 100X, the most sensitive taxonomic group was tested. If sensitivities were similar, both mysid and fish tests were conducted. Tissues from animals exposed in toxicity tests or in bioconcentration tests were analyzed for those pesticides whose partition coefficients indicated that they would be accumulable.

Since the environmental fate of a pollutant determines its availability to aquatic organisms, persistence studies have been included to complement the toxicity data on DEF, EPN, methyl parathion, carbophenothion, and phorate. These studies included the determination of the *n*-octanol/water partition coefficient (Log P) and the half-life or rate of disappearance of the pesticide under estuarine conditions. The Log P value has become the cornerstone for predicting the fate of organic chemicals from physical properties through the use of structure-activity correlations, because it is a potential predictor of bioaccumulation (Veith et al., 1979b), solubility (Chiou et al., 1977), sorption (Karickhoff et al., 1979), and persistence (Yonezawa and

Urushigawa, 1979). Half-lives of pesticides in aquatic systems, particularly in estuaries, are not available and are necessary for regulatory agencies to identify situations potentially detrimental to man and the environment.

The fate of methyl parathion has been extensively studied by us because we consider it to be a representative organophosphate insecticide and use it to calibrate the natural processes affecting the pesticide in a variety of microcosms under development at Gulf Breeze (Bourquin et al., 1979). These microcosms represent an alternative method to field studies for validating routine laboratory studies and serve to identify potential pesticide persistence or to isolate natural processes that contribute to pesticide dissipation (Pritchard et al., 1979).

METHODS AND MATERIALS

TEST ORGANISMS

All test organisms, except the oyster (Crassostrea virginica), post-larval penaeid shrimp (Penaeus stylirostris), and Atlantic silversides (Menidia menidia), used in toxicity tests described herein were either cultured at ERL,GB or BMRL, or collected from estuarine areas adjacent to these laboratories. The alga (Skeletonema costatum) was cultured at ERL,GB. Mysid shrimp (Mysidopsis bahia) and sheepshead minnows (Cyprinodon variegatus) used in acephate studies were cultured at BMRL. Those used in the carbophenothion, EPN, methyl parathion, or phorate studies were cultured at ERL,GB. Adult oysters were collected from Ocean Springs, Mississippi, and conditioned at BMRL in flowing, unfiltered seawater. P. stylirostris postlarvae were purchased from Ralston Purina's Marine Research Center, Crystal River, Florida; Atlantic silversides embryos were shipped from Wadmalaw Island, South Carolina. Tables 1-a, 1-b, and 1-c describe the species used, the chemicals tested, and the researcher responsible for each test at ERL,GB, as well as those tests conducted at BMRL.

All foods given to test animals during culture, holding, and testing at ERL,GB were analyzed chemically for chlorinated hydrocarbon content and contained less than 0.1 µg/g (micrograms per gram, wet weight). Mysid shrimp were fed living Artemia sp. nauplii. P. pugio were fed living Artemia sp. nauplii and a dry, flake food (nauplii as larvae and both foods as juveniles and adults). P. stylirostris were fed Artemia nauplii and a pelleted experimental food formulated and supplied by Ralston Purina. Pink shrimp (Penaeus duorarum) were fed grouper filets, and fish were fed either dry flake food, frozen adult Artemia, or live Artemia nauplii.

TEST PESTICIDES

Descriptions of the pesticides tested at ERL,GB and BMRL are contained in Appendix A. Concentrations of each pesticide are reported here as micrograms (µg) of pesticide per liter (ℓ) of seawater (parts per billion) or µg of pesticide per gram (g) of tissue, wet weight (parts per million).

TEST WATER

ERL,GB

All seawater used for culture and testing at ERL,GB (except for algal studies) was pumped by #316 stainless steel pumps from Santa Rosa Sound, Florida, through a coarse sand filter and polypropylene filters (10 to 20 micrometers [µm] pore size) into the laboratory. Seawater was then adjusted to the appropriate test temperature in reservoirs located near the test apparatus. From the reservoirs, seawater flowed by gravity to the various test systems. For algal studies, Rila Salt Mix (Rila Products, Teaneck, NJ) with nutrients added was the artificial sea salt medium.

BMRL

All water used for holding, acclimation, and testing was natural seawater pumped from Big Lagoon, Florida. The pump intake was located about 80 meters (m) from shore, at a depth of approximately 3 m.

Test water was pumped by a #316 stainless steel pump through hard polyvinylchloride (PVC) pipe, a fiberglass, sandfilled filter, and 10 µm polypropylene-core filters into an elevated fiberglass reservoir. Water was aerated continuously and vigorously in the reservoir and flowed by gravity through PVC pipes into the laboratory and the test systems. The salinity of water in tests was that of the incoming water.

ACUTE TOXICITY TESTS

Static Algal Tests

The chain-forming marine and estuarine diatom (Skeletonema costatum) was exposed to the eight pesticides in static tests as described by Walsh and Alexander (1980). Stock solutions of the pesticides in nanograde acetone were diluted with acetone to concentrations used in the tests. Since the pesticides were not equally toxic, concentrations differed for each, but working solutions were adjusted so that when 0.1 mℓ was added to 26 mℓ of test medium, desired exposure concentrations were obtained.

Since toxicity of the pesticides to algae was not known, it was necessary to expose S. costatum to a broad range of concentrations before definitive growth tests were done. Six concentrations between 1 and 10,000 µg/ℓ were used in range-finding tests. Concentrations used in definitive growth tests were based upon results of range-finding tests. Algae were exposed to at least five concentrations in definitive tests. Range-finding growth tests were done once; definitive tests were done three times. In each test, each concentration was replicated three times; therefore, nine values were obtained for each exposure concentration prior to calculation of the final EC50 (the calculated concentration that would inhibit growth by 50% in relation to control cultures). Growth of three control cultures was also measured in each test.

Biomass was estimated by optical density at 525 nm after growth for four days. The optical density data (Appendix C-1) were used to calculate the EC50 by nonlinear least squares analysis according to the expression

$$Y = A - \frac{A}{1 + P_1 X - P_2}$$

where

Y = estimated population density over the range of X,
X = range of concentrations,
A = mean maximum biomass,
P_1 = downward slope, and
P_2 = inflection point of downward slope.

Static Oyster Larval Tests

Methods for the 48-h oyster larvae toxicity tests were based on those of Woelke (1972) and American Society for Testing and Materials Committee E-35 on Pesticides (1978).

For the larval eastern oyster (*Crassostrea virginica*) studies, individual sexually mature females, held in glass chambers that contained 1 ℓ of filtered (5 μm) seawater, were induced to spawn by increasing the water temperature from 29 to 35°C. Viable sperm excised from the gonad of a sexually mature male oyster were added to each chamber. Fertilization occurred upon release of the eggs into the spawning chambers and was confirmed microscopically. Fertilization success was 91%. Population density of the embryos was determined by a Sedgwick-Rafter count of 1:10 dilution (1 mℓ embryo suspension to 10 mℓ seawater) of water from the spawning chamber.

Concentrations for definitive tests were based on the results of 48-h range-finding tests. All concentrations and the control were in triplicate. Test containers were 1 ℓ glass jars, each of which contained 900 mℓ of filtered (5 μm) natural seawater. Test concentrations were prepared by adding appropriate weighed amounts of the pesticide to each test container. If a solvent/carrier was required, the pesticide was dissolved in reagent grade triethylene glycol (TEG) and the appropriate amounts added to the test containers. A solvent control was also maintained to which was added the maximum volume of TEG.

Each test container was inoculated with an estimated 30,000 embryos within 1 h after fertilization, then maintained at 25 ± 1°C in a light- and temperature-controlled environmental chamber.

After 48-h exposure, the larvae from each container were collected and preserved separately in a constant volume of filtered seawater with neutralized formalin. The number of normally developed 48-h larvae was determined by a Sedgwick-Rafter count from each triplicate test concentration container and each control container.

Percentage reduction of normal embryos was determined as:

$$\text{Percentage reduction} = \frac{\text{Number of normal 48-h control embryos minust the number of normal 48-h embryos in each test concentration}}{\text{Number of normal 48-h control embryos}} \times 100$$

Static Copepod Test

A static, 96-h toxicity test was conducted with the copepod (*Acartia tonsa*) exposed to methyl parathion. The test was conducted in 50 x 90 mm glass crystallizing dishes, each containing 100 mℓ of test solution and 10 adult test animals. Test concentrations and the control were in triplicate, resulting in 30 animals per treatment. Salinity was 22 ^{0}/oo, and temperature was maintained at 22 ± 1^{0}C.

Based on results of a range-finding test, copepods were tested at nominal concentrations of 13 to 100 µg/ℓ. Test concentrations were prepared by pipetting appropriate weighed amounts of methyl parathion, dissolved in TEG, into each container. A solvent control was also maintained, to which was added the maximum volume of TEG (0.1 mℓ) that had been added to the treatment test containers. The seawater control received neither test material nor TEG.

Static Invertebrate and Fish Tests

Test methods used for static 96-h toxicity tests with mysid shrimp, penaeid shrimp, and fish were generally those of The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). The pesticides tested and species exposed are shown in Table 1-a. Stock solutions of each pesticide, except acephate, were prepared by dissolving appropriate quantities of each in TEG. Acephate was dissolved in distilled water. By using Hamilton^R microliter syringes, stocks were added directly to filtered seawater in jars or culture dishes. Solutions were stirred with ten vigorous swirls with a glass rod and allowed to equilibrate in an incubator or water bath for 30 minutes before addition of test animals. Twenty animals were exposed in each concentration of insecticide and in each of two control groups. One control contained seawater and TEG carrier at the same concentration as those used in the test solution chambers; the second control, seawater only. Juvenile *M. bahia* less than 24-h-old and *P. stylirostris* postlarvae were fed 48-h-old *Artemia* larvae during the tests to limit cannibalism or starvation. Mortality was recorded daily, and dead animals were removed when discovered. Test temperature for all studies was 25 ± 1^{0}C; test salinity, 20 ^{0}/oo. Dissolved oxygen values and pH, where monitored, are in Appendix C-8.

Flow-through Macrocrustacean and Fish Tests

Methods for all 96-h flow-through tests were those described by the Committee on Methods for Toxicity Tests with Aquatic Organisms

(1975). Five concentrations and a seawater control were used for acephate, aldicarb, carbophenothion, ethoprop, methyl parathion, and phorate. At least four concentrations and a seawater control were used for EPN and DEF. A TEG solvent control and a seawater control were used in studies on carbophenothion, EPN, methyl parathion, and phorate. Selection of concentrations for each test were generally based on the results of 96-h static tests. Pesticides studied, the species tested, and the laboratory and researcher conducting each study are listed in Table 1-b. Dissolved oxygen values and pH, where monitored, are in Appendix C-1.

The acephate tests were conducted in an open diluter constructed to deliver 1.0 ℓ/cycle/test aquarium at a dilution rate of 60%. Each test aquarium contained 42 ℓ of control seawater or test solution; the average number of cycles was approximately 6 per h, providing 3 volume additions every 24 h. The stock solution, prepared with deionized water, was delivered to the chemical mixing chamber by a 15-mℓ glass "dipping bird" to produce 5 test concentrations.

To begin the acephate test, the diluter cycled for about 24 h to equilibrate the system. Then, 10 fish were placed in each duplicate test aquarium, i.e., 20 fish per treatment. Salinity, temperature, dissolved oxygen (DO) concentrations, and pH were measured periodically during each test. A 450-mℓ water sample was taken on Day 1 and 4 from each duplicate aquarium, and samples for each day were combined in amber-glass bottles. Samples were preserved with 15 mℓ of chloroform ($CHCl_3$) for later chemical analysis.

Aldicarb tests were conducted in a closed diluter designed to deliver at a dilution rate of 60%. The test species and test aquaria volumes were: *M. bahia*, 8.5 ℓ; *P. duorarum*, 8.5 ℓ; *C. variegatus*, 53 ℓ; and *L. rhomboides*, 52 ℓ. At least eight volume additions per day were provided for the crustacean tests; at least two for the fish test. Triethylene glycol was used as a solvent.

Flow-through acute toxicity tests with carbophenothion were conducted by BMRL on the following species: *M. bahia*, *C. variegatus*, and *L. rhomboides*. An open diluter (1 ℓ/cycle/42 ℓ test aquarium) was used for the mysid test; closed, 1-ℓ diluters were used for the fish studies. Aquaria volume for the *C. variegatus* test was 53 ℓ; that for the pinfish study was 52 ℓ.

All flow-through acute toxicity studies with ethoprop were conducted by BMRL, using closed diluters. At least 2 volume additions were provided daily to each 52- or 53-ℓ test aquarium. Solvent for all tests was TEG.

All remaining flow-through penaeid shrimp and fish tests were conducted at ERL,GB with the pesticides EPN, carbophenothion, DEF, methyl parathion, and phorate. Appropriate quantities of the pesticides, dissolved in TEG, were metered by syringe pumps at 10 mℓ/ day into the filtered seawater that entered each aquarium from glass siphons calibrated to deliver 30 ℓ/h. One control aquarium received the same quantities of seawater and solvent but no insecticide; a second received only seawater. Twenty animals per aquarium were exposed to each concentration for 96 h. Mortality was recorded daily, and dead animals were removed when discovered. Fishes and pink shrimp that survived the EPN test were rinsed with acetone and analyzed for whole-body residues.

Flow-through toxicity tests with mysid shrimp, because of the shrimp's small size, required several modifications in the above procedures (Nimmo et al., 1977). Tests with acephate, aldicarb, carbophenothion, ethoprop, and methyl parathion used the diluters previously described. Tests with DEF, EPN, and phorate used syringe pumps and calibrated siphons described earlier for fish and shrimp tests with these pesticides. DEF, EPN, phorate, and methyl parathion were dissolved in TEG or acetone; therefore, a solvent control was provided. Juvenile mysids were placed in test containers that consisted of glass Petri dishes to which a 150-mm-high nylon screen collar (210 to 315 μm mesh opening) was attached with silicone sealant. Test containers were placed in each aquarium, resulting in 20 mysids per treatment. Self-starting siphons in the aquaria caused water depth to fluctuate, ensuring an exchange of water in containers. Mysids were counted by gently lifting containers from the aquaria, draining water through the nylon screen to the depth of the Petri dish, and placing them on a back-lighted table. Mysids were fed 48-h-old *Artemia* nauplii.

When dissolved oxygen concentrations in water of static and flow-through tests were less than 50% saturation--a violation in the procedures in the method stipulated by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)--data were presented in Tables 4, 5, and 8, but were appropriately footnoted. Several LC50 values in Tables 4, 5, and 8 were from tests in which species recommended in the method were used, but control mortality was excessive. *Penaeus stylirostris* was not a species recommended by the Committee.

CHRONIC TOXICITY TESTS

Mysid Shrimp Entire Life-cycle Test

Acephate test (BMRL)

The acephate entire life-cycle test was conducted in an open diluter constructed to deliver 0.5 ℓ/cycle/test aquarium at a dilution rate of 60%. The stock solution (1.932 g technical acephate/ℓ deionized water) was delivered to the chemical mixing chamber by a 13.2-mℓ, glass "dipping bird" to produce five nominal test concentrations that ranged from 1,300 to 10,000 µg/ℓ. A seawater control was also maintained; there was no solvent control.

Aldicarb Test (BMRL)

The aldicarb test was conducted in a closed diluter constructed to deliver 0.5 ℓ/cycle/test aquarium at a dilution rate of 60%. The stock solution, prepared with reagent grade TEG (19.5 mg technical aldicarb/100 mℓ TEG), was delivered to the chemical mixing chamber by a 50-mℓ glass syringe to produce five nominal test concentrations that ranged from 0.6 to 5.0 µg/ℓ. A seawater control and a solvent control were maintained.

Ethoprop Test (BMRL)

The ethoprop test was conducted in a closed diluter constructed to deliver 0.5 ℓ/cycle/test aquarium at a dilution rate of 60%. The stock solution, prepared with reagent grade TEG (10.0 mg technical ethoprop/100 mℓ TEG), was delivered to the chemical mixing chamber by a 50-mℓ glass syringe to produce five nominal test concentrations that ranged from 0.3 to 2.0 µg/ℓ. A seawater control and a solvent control were maintained.

In all aforementioned tests, the diluter cycled for about 24 h to allow the system to equilibrate before adding test animals. The test aquaria contained approximately 8 ℓ of control seawater or test solution; the average number of cycles was approximately 6/h, providing ≥ 9 volume additions every 24 h.

To begin the test, five mysids, 34- to 48-h-old, were placed in each test container and the containers were placed in the aquaria. The containers were glass Petri dishes to which a 15-cm-high nylon screen collar (Nitex^R HC-315 µm mesh opening) was attached with silicone sealant. Two test containers were placed in each of two sections of one aquarium of all treatments, providing duplicates (a total of 20 mysids). Recorded during the test were number of dead mysids, time to formation of brood pouches, and number of offspring. Mysids were fed 48-h-old *Artemia* nauplii *ad libitum*, daily throughout the test. After hatching occurred, a maximum of 10 juvenile progeny were isolated within each duplicate test container for continued exposure and observation. To observe mysids in the test containers, each container was lifted gently from the aquarium, the water allowed to drain through the screen to the top level of the Petri dish, the container then placed on a back-lighted table, and the number of living animals counted. Duration of these tests was 28 days.

Salinity, temperature, DO concentrations, and pH were measured periodically during each test. A 450-mℓ water sample was taken from each duplicate for chemical analysis on Days 1, 7, 14, 21, and 28 of each test. The two samples from each treatment were combined in an amber-glass bottle and preserved with 15 mℓ of chloroform ($CHCl_3$) for later chemical analysis.

EPN, Phorate, Carbophenothion, and DEF Tests (ERL,GB)

Test methods for the mysid EPN, phorate, carbophenthion, and DEF entire life-cycle tests were those of Nimmo et al. (1977). These tests employed syringe pumps and calibrated siphons. EPN, phorate, and DEF dissolved in a TEG carrier were metered by syringe pumps into seawater at 10 mℓ/day. Carbophenothion dissolved in TEG was metered into seawater at 5 mℓ/day.

The seawater, delivered by calibrated siphons, was mixed with the dissolved toxicant and entered each experimental or control aquarium at the rate of: 24 ℓ/h for the EPN and phorate tests, 25 ℓ/h for the carbophenothion test, and 30 ℓ/h for the DEF test. Two controls were provided for each test (one control with and one control without TEG carrier). TEG carrier concentrations were: 0.017 mℓ/ℓ for EPN and phorate, 0.008 mℓ/ℓ for carbophenothion, and 0.014 mℓ/ℓ for the DEF test. Nominal toxicant concentrations selected were: 0.075, 0.1, 0.3 and 3.0 µg/ℓ for EPN; 0.03, 0.08, 0.16, and 0.32 µg/ℓ for phorate; 0.18, 0.375, 0.75, 1.5, 3.0, and 6.0 µg/ℓ for carbophenothion; and 0.5, 1.0, 2.0, and 4.0 µg/ℓ for DEF.

The number of water additions per day for each test was: 12 for carbophenothion, EPN, and phorate tests; ≥14 for the DEF test; and 8 for the methyl parathion test.

Methyl Parathion Test (ERL,GB)

The methyl parathion test employed a 1-ℓ diluter similar to that of Mount and Brungs (1967) that delivered an intermittent flow of test water to the test chambers. Methyl parathion was dissolved in three parts TEG, and one part deionized water mixed with seawater, and delivered to the test chambers about every four minutes. Two controls were provided for the methyl parathion test (one with and one without the TEG carrier at 0.016 mℓ/ℓ). Nominal toxicant concentrations selected for this test were: 0.095, 0.19, 0.375, and 0.75 µg methyl parathion/ℓ seawater.

In each test (EPN, phorate, carbophenothion, DEF, and methyl parathion) as each test chamber achieved its maximum volume, a self-starting siphon drained the water to a volume of 1 ℓ. Fluctuating levels of seawater ensured an exchange of water within each aquarium and test container. Toxicant-laden test water was introduced into the test aquaria 24 h in advance of test animal exposure.

To begin each test, forty 24- to 48-h-old juvenile mysids (5 per test container) were placed in each concentration of toxicant and the two controls. The test containers were 100-mm glass Petri dishes to which a 15-cm-high nylon screen collar (Nitex® HE-210 μm mesh) was attached with silicone sealant. Mysids were fed 48-h-old *Artemia* nauplii *ad libitum* daily throughout the test. Duration of the tests was 28 days, except for methyl parathion which was 24 days. During this time, juveniles matured, became sexually active, and females produced multiple broods.

The total number of live mysids (by sex) and the number of progeny produced in each test container were recorded daily; dead mysids and progeny produced were removed daily. A bottom-lighted table was used to make daily observations of the test animals. Test containers were cleaned or replaced, as required, to ensure a proper exchange of seawater through the nylon mesh collars throughout the test.

One-liter water samples taken weekly from each test chamber and controls were analyzed for pesticide content.

Grass Shrimp Partial Life-cycle Toxicity Test

Test methods used for the grass shrimp carbophenothion partial life-cycle test were modified from those of Tyler-Schroeder (1978a, 1979). Laboratory-reared juvenile shrimp (12 to 19 mm rostrum-telson length) were used in the partial life-cycle test. Ovigerous females were collected from a natural population near ERL,GB. Larvae were hatched and reared to the onset of sexual maturity (approximately 1.5 months) in a flow-through system, using filtered, ambient seawater from Santa Rosa Sound. Photoperiod during this time was 4 to 6 h and was maintained, using 15-watt incandescent bulbs (Tyler-Schroeder, 1978b).

All exposures were conducted in a flow-through system, using a modified Mount and Brungs diluter (Mount and Brungs, 1967) to deliver 0.5 ℓ/cycle/tank, with a dilution ratio of 50%. The number of cycles averaged 518/day. The exposure aquaria contained approximately 57 ℓ and there were 4.5 volume additions every 24 h. The 8 test aquaria were 40.6 cm wide x 15.2 cm deep x 91.4 cm long. Egg trays used in the reproductive portion of the test were 29.8 cm wide x 13.3 cm deep x 55.9 cm long and rested on two strips of glass glued to the inside of the test aquaria. Stock solutions of carbophenothion were prepared in triethylene glycol (TEG) to give nominal concentrations of 0.125, 0.25, 0.50, 1.0, 2.0, and 4.0 μg carbophenothion/ℓ water. There was also a seawater and a carrier control. The highest test concentration was essentially the same as the 96-h LC50.

Grass shrimp were exposed to carbophenothion in a partial life-cycle to determine its effect on all aspects of development. One hundred laboratory-reared juveniles (12- to 19-mm rostrum to telson length) were randomly distributed to each test aquarium. The parental generation was observed daily, dead shrimp removed, and population counts were made on Days 1, 33, and 249 (test termination).

The light regime for the first two weeks was 8-h, provided by 15-watt incandescent bulbs. After this period, to allow for uptake of chemical by the shrimp, spawning was induced by increasing photoperiod to 10-h, provided by 100-watt incandescent bulbs. The light regime was increased further by a 47-minute increment at 2-week intervals until a regime of 14 h light:10 h dark was reached.

To determine the effects of carbophenothion on reproduction, the number of spawning females per concentration was recorded daily. The first 10 mothers were stripped of their eggs to determine individual production. The next ten ovigerous females were placed in a hatching apparatus (Tyler-Schroeder, 1979) that emptied into individual containers. These containers were constructed of 1-ℓ heavy duty beakers with a 4 cm diameter hole approximately 1 cm from the bottom and covered with 363 μm Nitex^R screen, similar to those described by Buchanan et al. (1975). A self-starting siphon in each egg tray provided the fluctuating water levels which ensured an exchange of exposure medium in each beaker.

Larvae that hatched into beakers were exposed throughout their development to the juvenile stage to the same concentration as their parents. They were counted and fed fresh Artemia nauplii daily. As larval survival was seemingly affected by test conditions other than toxicant concentration (i.e., wide salinity fluctuations, low dissolved oxygen, and presence of hydrogen sulfide), multiple replicates of larval survival were run in each concentration. Ultimately, survival data from only the first 10 days of exposure were used.

After 35 days of exposure during larval development, the rostrum-telson lengths of the postlarval shrimp were measured. Postlarval exposure to carbophenothion was continued past Day 35 to test termination. Because of differences in hatch times, postlarvae at test termination varied in age from 45 to 78 days. The age was noted for each group and used in the statistical analysis.

At test termination, shrimp from parental and F_1 generation populations were measured individually (rostrum to telson length). All shrimp from each exposure concentration were sacrificed and weighed together, then frozen and placed in glass tubes for residue analysis.

Sheepshead Minnow Embryo/Juvenile Tests

Twenty-eight day embryo-juvenile toxicity tests were conducted with the insecticides aldicarb, carbophenothion, ethoprop, and phorate to determine effects on embryonic development, hatching success, survival, and growth of hatched sheepshead minnows (Cyprinodon variegatus).

Aldicarb and Ethoprop Tests (BMRL)

Sheepshead minnow embryos for the aldicarb and ethoprop embryo-juvenile tests were obtained from fish collected from Big Lagoon, a Gulf of Mexico estuary adjacent to BMRL. Eggs were obtained from females whose egg production was enhanced by injections of human chorionic gonadotrophic hormone on two consecutive days. The eggs were fertilized by the addition of a sperm suspension made from macerated testes excised from adult male fish.

Both tests were conducted in an intermittent-flow system by using a proportional diluter (Mount and Brungs, 1967) constructed to deliver 1 ℓ/cycle/test aquarium at a dilution rate of 60%. For each test, a stock solution of the pesticide was delivered into a mixing chamber where it was diluted, then siphoned down into the chemical cells. A Teflon solenoid valve, controlled by a float switch, regulated seawater flow into the water cells. Mixing of uncontaminated dilution seawater with test solution in the chemical cells produced the five concentrations of test solution that were distributed to the appropriate test chambers.

Five concentrations, a control, and a solvent control were all duplicated. Selection of concentrations was based on the results of 96-h flow-through tests.

Light was provided by two 3.7-m fluorescent bulbs suspended 53 centimeters (cm) above the test containers, providing approximately 1,300 lux incident to the water surface. Photoperiod was 12 h.

To begin each test, the diluter cycled for about 4 days to permit system equilibration. Eggs and sperm were obtained as previously described, and within 4 h after visual confirmation of fertilization, two groups of 50 embryos each per treatment were placed randomly in incubator cups (Pyrex® tubing 51 mm in diameter and 75 mm in length with 315 μm square mesh nylon screen attached to one end with silicone sealant). The incubator cups were placed into embryo cup holding chambers equipped with automatic siphons which allowed the holding chambers to fill and empty with each diluter cycle. Embryos were removed from each cup by pipette daily, counted, and the cups washed with seawater to clean the screens. This procedure was repeated until all living embryos had hatched. Embryo mortality and time to hatch were recorded. After hatch, 40 juveniles from each replicate were impartially selected and placed in glass chambers (14 cm wide x 20.5 cm high x 26 cm long with 480 μm square mesh nylon screen over one end). Juveniles were maintained in the growth chambers until the end of the test and fed live brine shrimp (Artemia salina) nauplii daily. Survival was monitored daily for 28 days posthatch and any changes in physical appearance of the fish or changes in behavior were recorded. Growth of juveniles (standard length) was determined photographically at the end of the exposure. All surviving fish were collected, pooled according to treatment, wrapped in aluminum foil, placed in a glass jar, and frozen for later chemical analysis.

Diluter function was checked daily by observation and weekly by measurement of toxicant concentration. Water samples were collected every 7 days, starting on Day 1; 450-mℓ samples were taken from both replicates and combined for each treatment.

Salinity and temperature were measured daily, except as noted. The pH and DO concentrations were measured in one duplicate set of test containers daily, except as noted.

Test chambers contained approximately 50 ℓ of control seawater or test solution and there were >2 volume additions every 24 h. The stock solution 0.62 g aldicarb/ℓ of TEG or 7.0 g ethoprop/ℓ TEG was delivered to the chemical mixing chamber by a 50-mℓ glass syringe to produce the five nominal test concentrations that ranged from 17 to 134 µg aldicarb/ℓ or 26 to 200 µg ethoprop/ℓ. Another syringe containing only TEG provided the test with a solvent control.

Carbophenothion and Phorate Tests (ERL,GB)

The exposure apparatus used for both studies was a modified Mount and Brungs (1967) diluter similar to that used by Schimmel et al. (1974). Seawater used in these studies was filtered (≥20 µm), heated to 30 ± 2°C and aerated before delivery to the diluter.

Adult sheepshead minnows were collected near ERL,GB and acclimated to 30°C at ambient salinity for 5 days prior to hormonal enhancement of egg production. Female sheepshead minnows (10 for the carbophenothion test, 12 for the phorate test) were injected intraperitoneally with 50 I.U. of human chorionic gonadotrophic hormone on two consecutive days, then manually stripped of eggs on the second day following the final injection. Eggs were fertilized in approximately 100 mℓ of 30°C filtered seawater with sperm from macerated testes from 9 male fish and held at 30°C for one hour before distribution. Chemical analysis of excess eggs revealed no detectable chlorinated insecticides or polychlorinated biphenyls (<0.05 µg/g carbophenothion; <0.02 µg/g phorate).

In the carbophenothion test, the diluter cycled an average of 278 times per day, delivering 500 mℓ of exposure water to each of two 3.2 ℓ aquaria per concentration per cycle. A carbophenothion stock solution was prepared in triethylene glycol to give nominal exposure concentrations of 0.38, 0.75, 1.5, 3.0, 6.0, and 12.0 µg of carbophenothion/ℓ of seawater. Average measured concentrations and standard deviations in weekly composite water samples were: non-detectable (control = <0.05 µg/ℓ), 0.36 ± 0.053, 0.59 ± 0.015, 1.3 ± 0.082, 2.8 ± 0.34, 5.4 ± 0.33, and 10.8 μg/ℓ ± 1.3 (n = 4 in all cases except 10.8 μg/ℓ where n = 3). Triethylene glycol was delivered to each toxicant concentration and the control at a concentration of 8.0 mg/ℓ of seawater.

In the phorate test, the diluter cycled an average of 243 times per day, delivering 500 mℓ of exposure water to each of two 3.2 ℓ aquaria per concentration per cycle. A stock solution was prepared in TEG to give nominal concentrations of 0.094, 0.19, 0.38, 0.75, 1.5, and 3.0 μg of phorate/ℓ of water. Average measured concentrations and standard deviations in weekly composite water samples were: non-detectable (control = <0.02), 0.16 ± 0.04, 0.24 ± 0.06, 0.41 ± 0.18, 0.77 ± 0.29, 1.2 ± 0.60, and 2.4 μg/ℓ ± 0.49 (n = 4, except control, where n = 5, and 2.4 μg/ℓ, where n = 2). Solvent was delivered to each toxicant concentration and the control at a concentration of 8.0 mg of TEG/ℓ of seawater.

In each test, one hour after fertilization, 20 embryos were distributed randomly in egg cups (Petri dishes with a 9 cm collar of 450 μm nylon mesh). Distribution in the test system was: two egg cups per aquarium, two aquaria per concentration, and six toxicant concentrations and a control with carrier, or a total of 28 egg cups. To ensure exchange of water in each egg cup, self-starting siphons produced 5 cm fluctuations of water levels in aquaria approximately 60 times/day in the carbophenothion study; 18 times/day in the phorate study. Dissolved oxygen, measured weekly by the modified Winkler method of Strickland and Parsons (1968), remained above 50% saturation in all aquaria throughout each test. Salinity averaged 18.9 ^{o}/oo in each test (range 8 to 28 ^{o}/oo in the carbophenothion test, 6.5 to 28 ^{o}/oo in the phorate test). Aquaria were partially submerged in a recirculating water bath that maintained the exposure temperature at 30 ± 2°C. Photoperiod was 12 h. After hatching, fish were fed live *Artemia salina* nauplii that contained no detectable (<0.05 μg/g) carbophenothion and no detectable (<0.02 μg/g) phorate, chlorinated insecticides, or PCBs.

Embryos and hatched fish were observed daily for mortality and signs of poisoning. On Day 28, fish in each egg cup were photographed for measurement of standard length. Fish from each treatment in the carbophenothion test were preserved for pathological examination and the remaining fish from 0.59, 1.3, and 2.8 μg/ℓ were sacrificed by immersion in hot water, towel-dried, weighed, and frozen for later chemical analysis. Fish in the phorate study were not preserved for pathological examination, but were analyzed for phorate content.

Ten fish each from control, 0.36, 0.59, 1.3, and 2.8 μg carbophenothion/ℓ and four fish from 5.4 μg/ℓ were fixed in Davidson's fixative prior to being embedded, sectioned, and stained by the methods of Couch et al. (1979). Samples of fish were coded so the pathologist had no *a priori* knowledge of the sample's exposure history.

Therefore, samples were classified as effect or no-effect groups solely on histopathological findings.

Sheepshead Minnow Entire Life-cycle Test

A sheepshead minnow entire life-cycle toxicity test lasting 265 days was conducted with the insecticide EPN. Seawater was gravity-fed to a constant-head box, where it was heated to 30 ± 2°C and aerated. Ambient salinity water (average 24.9 °/oo, range = 6.5 to 32.5 °/oo) was then delivered intermittently to the fourteen 70-ℓ exposure aquaria by a 2-ℓ diluter similar to that of Schimmel et al. (1974). Water flow from the diluter was divided so that 1 ℓ was delivered to each of two duplicate aquaria in each of the seven treatments. The exposure temperature was maintained at 30 ± 2°C in an upper and a lower water bath; each bath contained one aquarium for each of the seven treatments. During each cycle of the diluter, 12 of the 14 aquaria received 1 ℓ of seawater containing EPN and 6.7 μℓ of the carrier solvent TEG. The two control aquaria received the same volume of seawater and TEG. The photoperiod was 12 h. Nominal EPN concentrations were: 0.31, 0.62, 1.2, 2.5, 5.0, and 10.0 μg/ℓ. Average measured EPN concentrations and standard deviations of weekly water samples in μg/ℓ were: nondetectable (control = <0.05); 0.25 ± 0.09; 0.50 ± 0.13; 0.88 ± 0.22; 2.2 ± 0.38; 4.1 ± 0.61; and 7.9 μg/ℓ ± 1.32 (n = 38 except in 4.1 and 7.9 μg/ℓ, where n = 37 and in 0.25 μg/ℓ, where n = 39).

The test began with embryos spawned from adult sheepshead minnows that were collected near EPA's Gulf Breeze Environmental Research Laboratory, maintained for 48 days at the same temperature and photoperiod used in the toxicity test, and injected with human chorionic gonadotrophin to enhance egg production, as in the embryo/juvenile fish study. Eggs from 17 females were incubated with macerated testes from eight males for approximately 1 h in approximately 100 mℓ of 30°C filter seawater. Chemical analyses of excess eggs revealed no detect- able EPN (<0.25 μg/g), organochlorine pesticides (<0.02 μg/g), or polychlorinated biphenyls (<0.25 μg/g).

Twenty fertile eggs were placed in each of three egg cups (9-cm I.D. glass Petri dish bottoms to which 10-cm-high 450 μm nylon mesh tubes were attached) per exposure aquarium. Egg cups were placed in trays within the aquaria that received water from the diluter; the water then flowed to the aquaria via self-starting siphons that caused the water level within the trays to fluctuate approximately 5 cm and vary the water volume in the tray from approximately 2.75 to 6.67 ℓ.

Embryos and hatched fish were observed daily for mortality and signs of poisoning. On Day 28, fish in each egg cup were photographed for measurement of length and some fish from each egg cup were removed and combined within duplicate treatments for analysis of EPN content. After replacing the self-starting siphon with a stand-pipe, ten fish from each of the three egg cups per duplicate aquarium were released into the tray in the aquarium. The trays were removed on exposure Day 49 and the fish were released into their aquaria. All fish were also photographed on Days 60, 88, 118, and 140.

We investigated the effect of EPN on sheepshead minnow reproduction in three spawning trials between exposure Days 150 and 231. After fish attained reproductive size ($\geq$26 mm S.L.), groups of three females and two males were placed in glass spawning chambers in each aquarium (345 x 190 x 230 mm high and a water depth of approximately 150 mm) (Hansen et al., 1977). The ends and bottom of the chambers were covered with 6.4 mm^2 mesh stainless steel screen that allowed eggs to fall onto a removable tray constructed of Plexiglas^R and 450 µm nylon mesh. Trials one and three lasted 10 days; trial two, 14 days. Spawning groups were monitored in each of the 14 aquaria in all spawning trials, except the third, where all spawning groups were not available. Eggs were removed daily, counted, and examined microscopically to determine percentage fertility. Adult fish from the first and third spawning trials were sacrificed for analysis of brain acetylcholinesterase activity and analysis of EPN content.

Where adequate numbers of eggs were obtained, we investigated the effect of EPN on progeny of adults that had been continuously exposed for 222 to 231 days by placing 20 fertile eggs in each of two egg cups contained in trays located in the same aquarium in which the eggs were spawned. Whenever eggs were not produced in one of the duplicates, but were available from the other, eggs were transferred between duplicates. Extra eggs from the spawning trials were combined within exposure concentration and frozen until analyzed for EPN. Embryos and hatched fish were observed daily for death and signs of poisoning. Fish were fed live brine shrimp nauplii. After a 28-day exposure, surviving fish were photographed for length measurement, combined within treatments, and frozen for later analysis of EPN content.

Benthic Animal Community Test

The effect of carbophenothion on colonization by macrobenthic organisms was determined by comparing numbers and species of animals that grew from planktonic larvae in contaminated aquaria for 8 weeks and 3 days (March 8 - May 7, 1979) with those of animals that grew in uncontaminated aquaria. Larvae entered the aquaria from the natural component of plankton in flowing unfiltered seawater. We used 4 plexiglass apparatuses (shown in Tagatz et al., 1979), each consisting of a central constant-head box and 8 aquaria (40 cm long, 10 cm wide, and 12 cm high). Aquaria were filled to a depth of 5 cm with clean silica sand (size range of 96% of the particles was 250-710 µm) dredged from Santa Rosa Sound, Florida. Water levels in aquaria were maintained at 8 cm (3 cm above substratum).

Seawater with its constituent plankton was pumped from Santa Rosa Sound to a splitter box, where 4 adjacent glass tubes supplied water at a rate of 2 ℓ/min to the 4 constant-head boxes that supplied water to the aquaria. Flow to each aquarium was maintained at 200 mℓ/min by adjusting the height of a 3-mm diameter hole in the wall of the constant-head box. Water flowed from each aquarium through a notched end-opening; large predators escaped through these openings before their numbers could drastically affect community structure.

Technical grade carbophenothion dissolved in triethylene glycol (TEG) was metered by syringe pump into and mixed with the seawater that entered the center of the constant-head box of each contaminated apparatus. The same amount of TEG was metered into the control apparatus. Desired concentrations of carbophenothion in seawater were 0.01, 0.1, and 1.0 μg/ℓ.

Samples of water were taken from constant-head boxes once a week for carbophenothion analyses. At the end of exposure, meats of the clam (Mulinia lateralis) were obtained from individuals in control, 0.01 μg/ℓ, and 0.1 μg/ℓ aquaria for carbophenothion analyses (pooled sample of 5 g for each treatment).

After 8 weeks and 3 days, animals were collected by siphoning the contents of the aquaria into a 1-mm mesh sieve, then preserved and identified.

Bioconcentration Tests

Carbophenothion

Juvenile spot (Leiostomus xanthurus, 24 to 36 mm standard length, $\bar{x}$ = 32.4 mm) were exposed to carbophenothion for 15 days followed by a 4-day depuration period. The exposure apparatus was that of Schimmel et al. (1977). A stock solution of carbophenothion dissolved in 95% TEG: 5% acetone was prepared at the beginning of the test; calculated deliveries were 5.0 and 50 μg carbophenothion/ℓ seawater. Each exposure concentration was duplicated, two control aquaria being provided, one with and one without TEG. We selected these concentrations because: (1) acute toxicity studies indicated that they were well below the carbophenothion lethal threshold for spot; and (2) they were quantifiable by gas chromatographic analyses.

Fifty-five juvenile spot were placed in each experimental and control aquarium at the beginning of the test. Three fish from each control, 5.0 and 50 μg/ℓ aquarium were sampled for bioconcentration at Days 1, 2, 4, 8, 11, and 15. The chemical exposure was then terminated and remaining fish were held in carbophenothion-free seawater and sampled as they depurated on Days 1, 2, and 4. Seawater temperature averaged 24.6°C (range = 24-25°C); seawater salinity averaged 23 ‰ (range = 16 to 30 ‰).

EPN

Juvenile pinfish (44 to 77 mm standard length, $\bar{x}$ = 59 mm) were exposed to EPN for 26 days, followed by an 8-day depuration period. The exposure apparatus was that used in the carbophenothion test (Schimmel et al., 1977). A stock solution of EPN dissolved in TEG was prepared at the beginning of the test; calculated deliveries were 0.25 and 2.5 µg EPN/ℓ seawater. Each exposure concentration was duplicated, two control aquaria being provided, one with and one without TEG. We selected these water concentrations, using the same criteria as those in the carbophenothion test.

Thirty juvenile pinfish were placed in each experimental and control aquarium at the beginning of the test. Two fish from each control, 0.25, and 2.5 µg/ℓ aquarium were sampled for bioconcentration at Days 1, 2, 4, 8, 15, 18, and 22. The chemical exposure was terminated after 26 days, and remaining fish were held in EPN-free seawater and sampled as they depurated on Days 1, 2, 4, and 8. Tissues were analyzed on a whole-body, wet-weight basis. Seawater temperature averaged 25°C (range = 23 to 28°C); seawater salinity averaged 25.7 °/oo (range = 22 to 31 °/oo).

PERSISTENCE

Log P

The octanol/water partition coefficient was calculated using retention time on a high pressure liquid chromatograph (HPLC) (Veith, 1979a). The instrument was a Waters Associates High Pressure Liquid Chromatograph with a Model 440 Absorbence Detector operated at 254 nm. The column was a Waters Associates Micro Bondapak C18 with the theoretical plates in excess of 4000. The solvent system was 20% water in methanol (v/v) at a flow rate of 1.0 mℓ/min. Column calibration standards were benzene (Rt 42 mm); biphenyl (Rt 65 mm); p,p'-DDE (Rt 168 mm); and hexachlorobenzene (Rt 224 mm). A plot of Log R_t for these standards versus Log P (n-octanol/water) was linear with r^2 for the correlation equal to 0.98. Using the equation for this relationship, the retention times for methyl parathion, phorate, EPN, DEF, and carbophenothion were determined by HPLC and converted to Log P.

Persistence Test

The persistence of EPN, methyl parathion, phorate, DEF, and carbophenothion was studied in the laboratory to resolve those processes in marine environments that contribute most to the pesticide's disappearance. The relative importance of nonbiological and biological processes was investigated by eliminating or amending the sediment and water components into three types of systems: one with water only (Seawater), a second with water and sterilized sediment (Sterile), and a third with water and unsterilized sediment (Untreated). Water was filtered (20 μm) natural seawater from Santa Rosa Sound; sediment (described in Table 3) was collected from a salt marsh adjacent to Range Point, Santa Rosa Island, Escambia County, Florida.

The experimental apparatus consisted of 150 mℓ Corex centrifuge bottles fitted with No. 6 Neoprene stoppers. A typical system contained 10 g (wet weight) of sediment and 100 mℓ of seawater (27 ^{o}/oo). Some of these systems were sterilized by addition of 5 mℓ of formalin to identify non-biological processes affecting pesticide persistence. A series of systems were prepared that contained only 100 mℓ of seawater to better resolve the role of sediments in pesticide persistence. Systems were fortified with 10 μg of pesticide delivered in 10 μℓ of acetone, stoppered, and aerated at 50 mℓ/min. Air exiting each system was passed through a column of Amberlite XAD-4 resin to trap vaporized pesticide. Systems were incubated at 25°C with 12-h photoperiod under G.E. White Fluorescent lights.

Duplicate unsterilized sediment and water systems were analyzed on Day 0 and 5; systems containing water or sterilized water and sediment were analyzed on Day 0 and 7. Contents of the bottles were centrifuged at 1,600 G and the water phase decanted into a separatory funnel. Water and sediment were extracted and analyzed by procedures in the analytical methods section. Pesticide was eluted from the resin with acetone and quantitated by procedures described in the analytical methods section.

In a separate experiment, the effect of sunlight on the persistence of methyl parathion was investigated. We added 300 mg of methyl parathion in acetone to a 4-ℓ amber bottle and purged the acetone with a gentle stream of nitrogen. Three ℓ of seawater were added to the bottle and it was agitated vigorously on a shaker at 25°C overnight; 100 mℓ aliquots were added to 250 mℓ Erlenmeyer flasks fitted with ground glass stoppers. Half of the flasks were covered with aluminum foil, and the other half were not covered. The systems were exposed outdoors to direct sunlight and sampled on Days 0, 3, and 7. Temperatures in the flasks varied from a high of 40°C during the day to a low of 22°C at night. Duplicate samples were analyzed each sampling day by procedures described in the analytical methods section.

PESTICIDE ANALYTICAL METHODS (BMRL)

The following methods apply only to toxicity testing conducted by BMRL, Pensacola on acephate, aldicarb, and ethoprop. These methods and the instrument parameters are detailed in Appendix B.

PESTICIDE ANALYTICAL METHODS (ERL,GB)

The following analytical methods apply only to toxicity and persistence testing conducted by ERL,GB on EPN, methyl parathion, phorate, DEF, and carbophenothion.

Water

One liter of seawater was extracted twice with 100 mℓ of petroleum ether. The combined extracts were collected in a Kuderna-Danish concentrator and evaporated on a steam table with a Snyder column to no less than 20 mℓ. For further concentration, samples were transferred to a nitrogen evaporator with a water bath maintained at 35°C and then analyzed.

Tissue

Phorate and Carbophenothion

One to ten grams of tissue were weighed into 150 mm x 25 mm screw cap test tubes and extracted four times with 5 mℓ portions of acetonitrile, using a Willems Model PT 10-ST polytron (Brinkman Instruments, Westbury, N.Y.). The test tube was centrifuged after each extraction and the combined extracts were diluted with 75 ml of 2% (w/v) aqueous sodium sulfate and extracted four times with 5 mℓ portions of hexane. The hexane extracts were combined in a 25 mℓ concentrator tube and evaporated with a gentle stream of nitrogen to 1.0 mℓ. The concentrate was transferred quantitatively to a Sep-Pak silica cartridge (Waters Associates, Milford, Mass.) with two 0.5 mℓ portions of hexane. Phorate or carbophenothion was eluted from the cartridge with 15 mℓ of 1% (v/v) diethyl ether in hexane and analyzed. Tissue concentrations were calculated on a wet weight basis.

EPN and DEF

Tissues were extracted similar to phorate except that the hexane concentrate was transferred to a 9 mm Chromoflex column (Kontes Glass Company, Vineland, N.J.) containing 3.0 gm of florisil topped with 2.0 gm anhydrous sodium sulfate. (The column had been previously washed with 10 mℓ of hexane.) Following the transfer of the extract, the column was eluted with 20 mℓ of 5% diethyl ether in hexane to remove PCBs and chlorinated pesticides. EPN or DEF was eluted with 25 mℓ of 10% isopropanol in isooctane and then analyzed.

Methyl Parathion

Tissues were extracted similarly to phorate, except that methanol was substituted for acetonitrile, and the aqueous sodium sulfate/methanol mixture was extracted with petroleum ether instead of hexane. Methyl parathion was eluted from the Chromoflex column described for

EPN tissues with 20 mℓ of 50% (v/v) diethyl ether in hexane and then analyzed.

Sediment

Ten grams of sediment (wet weight) were extracted four times with 25 ml of acetonitrile for 30 seconds with a Branson Sonifier Model 350. The combined extract was diluted with 1 ℓ of seawater and extracted according to the water procedure previously described. The concentrated extract was then cleaned, using the method previously described for tissue samples and then analyzed. Sediment concentrations were calculated on a dry weight basis.

ANALYTICAL EQUIPMENT AND QUALITY ASSURANCE

Instrumentation

Analyses were performed with Hewlett-Packard Model 5730A gas chromatographs, using either a Model 1879A dual nitrogen-phosphorus flame ionization detector or a Nickel-63 linear electron capture detector. Glass columns (182 cm by 2 mm I.D.) were packed with either 2% SP 2100 on 100/120 mesh Supelcoport or 5% QF-1 on 80/100 mesh Gas Chrom Q. Compounds were quantitated by peak area, using a Hewlett-Packard Model 3352B Laboratory Data System.

Quality Control

The lower limit of detection for EPN, methyl parathion, DEF, carbophenothion, and phorate in seawater was 0.02 µg/ℓ and in tissues and sediment, 0.02 µg/g, unless otherwise noted. Recoveries from fortified samples of seawater, tissues, and sediment were greater than 85%. Each set of samples analyzed included a fortified sample to evaluate recoveries for that day. Analyses were not corrected for percentage recovery of fortifications. The chemicals used in these studies are described in Appendix A and were positively identified by mass spectrometry, using a Finnigan Model 4000 EI/CI gas chromatograph mass spectrometer.

ACETYLCHOLINESTERASE (AChE)

Acetylcholinesterase activity in brains of adult sheepshead minnows continuously exposed to EPN was assayed by the pH-stat method of Coppage (1971). For each AChE determination, nine fish brains were divided into pooled samples of three brains each. Percentage AChE inhibition was determined by comparison with mean normal activity of control fish on each day of sampling.

STATISTICAL METHODS

Algal Test

Nonlinear regression procedures (Bahner and Oglesby, in press) were used to analyze the algal inhibition data. From the resulting equations and parameter estimates, an approximate EC50 and 95% confidence limits were established.

Oyster Embryo Test

Test concentrations from the oyster study were converted to logarithms and the corresponding percentage reduction of normal embryos was converted to probits to estimate the concentration that would in 48 h reduce the number of normal embryos by 50% as compared to the number of control embryos (48-h EC50) (Finney, 1971). The estimated 95% confidence limits for the 48-h EC50 were then obtained. To determine whether normal development of embryos in the solvent control differed from that of the control, data were analyzed by Student's t-test (Steel and Torrie, 1960). Differences were considered significant at $\alpha = 0.05$.

Acute Toxicity Test

Mortality data from acute toxicity tests were analyzed by the probit analysis method of Finney (1971), moving average, or binomial test to estimate concentrations of insecticide in water that killed 50% of the test animals (LC50) and the 95% confidence intervals for these concentrations. Abbot's correction was used in all cases where control mortality was observed (Finney, 1971).

Mysid Shrimp Entire Life-cycle Tests

Acephate, Aldicarb, and Ethoprop

At the termination of the acephate tests by BMRL, the differences among the percentage mortalities of control and exposed mysids were determined for adults for each 7-day period by analysis of variance (ANOVA), after an arcsin transformation (Neter and Wasserman, 1974). Statistical comparison between the control and each concentration was made by Williams' (1971) method. Significant differences in number of offspring produced in different treatments were determined by ANOVA and Williams' method. Differences were considered significant at $\alpha = 0.05$.

Carbophenothion, DEF, EPN, Methyl Parathion, and Phorate

One way ANOVA and various post hoc tests were used to determine if mortality or reproduction were significantly affected ($\alpha = 0.05$) in mysid entire life-cycle tests conducted at ERL,GB. In the DEF and carbophenothion studies, ANOVA with Duncan's procedure (Steel and

Torrie, 1960) was used to detect significant differences in survival and reproductive success. In the EPN study, ANOVA and Student-Newman-Keul's (SNK) test (Steel and Torrie, 1960) were used to detect significant differences in mortality while ANOVA and Dunnett's test (Steel and Torrie, 1960) were used to detect significant differences from control in reproductive success (mean number of progeny per female). In the methyl parathion study, significant differences in survival and reproductive success were detected by ANOVA and the SNK test. In the phorate study, ANOVA and SNK post hoc tests were used to detect significant differences in survival, and ANOVA with Duncan's multiple range test were used to detect significant differences in reproduction.

Grass Shrimp Partial Life-cycle Tests

Carbophenothion

The adult mortality data were analyzed at Day 33 and Day 249 (end of test) of the exposure. An LC50 was generated, by either the probit, binomial, or moving average method.

Spawning success was examined by comparing the total number of shrimp that had spawned in each test concentration on four different days during the test (Day 21, 84, 147, and 191), using a t-test with a Bonferroni adjustment for the number of test run (Neter and Wasserman, 1974).

Lengths and weights of parent and F_1 generation shrimp were analyzed by one-way ANOVA followed by Duncan's test to determine differences between means.

Possible effect of carbophenothion on fecundity (egg production and hatching efficiency) was determined by one-way ANOVA, using the length of the female producing the eggs or larvae as a single covariate. The length of female shrimp is related to the number of eggs produced, and, therefore, to the number of larvae to hatch (Jensen, 1958). A post hoc test was performed on adjusted means with an adjustment to the α level for the number of tests performed.

Sheepshead Minnow Embryo/Juvenile and Entire Life-cycle Test

At termination of the embryo/juvenile toxicity tests, the percentage survival of embryos to hatching, juvenile survival, overall survival, and average lengths were calculated. Except for phorate, all survival data were analyzed using analysis of variance (ANOVA). Aldicarb and ethoprop survival percentages were transformed using arcsins and differences between treatments were determined using Williams' test. Differences in survival between treatments in the carbophenothion test were determined using Duncan's test. Chi-square tests (Neter and Wasserman, 1974) were used to analyze phorate survival data. Analysis of variance and Duncan's test (carbophenothion) or Williams' test (aldicarb and ethoprop) were used to determine differences in growth. Analysis of covariance and Student-Newman Keul's test (phorate) or Duncan's test (Neter and Wasserman, 1974) (carbophenothion) were used to determine differences in growth. The covariate was the number of fish in a particular exposure chamber summed over the number of days over which length data were compared (fry-feeding-days, FFD).

At termination of the EPN entire life-cycle toxicity test, percentage survival of embryos and fish, average lengths, percentage fertility, average number of eggs spawned, and AChE levels were calculated. Survival data for embryos and 28-day-old fish from the parental and progeny generations and reproductive data were analyzed by ANOVA; other survival data were analyzed by chi-square tests. Growth data were analyzed by analysis of covariance using the covariate FFD and Duncan's test. AChE activity of fish exposed to EPN was compared with that of control fish by Student's t-test.

All tests were performed, using a level of significance of 0.05.

Bioconcentration Tests

The statistical model of Bahner and Oglesby (1979) was used to determine steady-state bioconcentration factors (BCFs = steady-state concentration in whole animals, wet weight, divided by concentration measured in exposure water) and to describe uptake and depuration of carbophenothion in spot and EPN in pinfish.

Persistence Tests and Log P

Log P was estimated according to the method of Veith et al. (1979b). A linear calibration of the logarithm of retention time (relative to methanol) with the logarithm of the partition coefficient (Log P) was determined using benzene, biphenyl, p,p'-DDE, and hexachlorobenzene. The correlation coefficient (r^2) was 0.98.

Persistence tests were run in duplicate, with the average values being reported.

RESULTS AND DISCUSSION

ACEPHATE

Results

Acute Static Toxicity Tests

Algae--The 96-h EC50 for *Skeletonema costatum* exposed to acephate was >50,000 µg/ℓ (Table 4-a and Appendix C-1); the no effect concentration was not determined.

Invertebrates--The toxicity of acephate to embryos of eastern oysters (*Crassostrea virginia*) is shown in Table 4-a, Appendix C-2a, and Appendix C-2b. The 48-h EC50 was 150,000 (8,000 to 300,000) µg/ℓ. The 96-h LC50 for juvenile pink shrimp (*Penaeus duorarum*) was >10,000 µg/ℓ (Table 4-a and Appendix C-3).

Fishes--The static acute toxicity expressed as the 96-h LC50s of acephate to sheepshead minnows (*Cyprinodon variegatus*) was >3,200,000 µg/ℓ and for spot (*Leiostomus xanthurus*) was >100,000 µg/ℓ (Table 4-a, Appendix C-4, and Appendix C-5).

Acute Flow-through Toxicity Tests

Invertebrates--The 96-h LC50 of acephate to mysid shrimp (*Mysidopsis bahia*) based on measured concentrations was 7,300 (300 to 190,000) µg/ℓ (Table 5-a and Appendix C-6). The 96-h LC50 of acephate to pink shrimp, based on nominal concentrations, was 3,800 (2,000 to 7,300) µg/ℓ (Table 5-a and Appendix C-7). Ranges of DO and pH measured in seawater used in these tests are given in Appendix C-8.

Fishes--The 96-h LC50 of acephate to pinfish (*Lagodon rhomboides*), based on nominal concentrations, was 85,000 (7,800 to 933,000) µg/ℓ (Table 5-a and Appendix C-9). Sheepshead minnows were affected by acephate and in some concentrations, completely lost equilibrium, but only one fish died in the highest concentration tested (1,100,000 µg/ℓ). The 96-h EC50, based on loss of equilibrium was about 910,000 µg/ℓ (Table 5-a and Appendix C-10). Ranges of dissolved oxygen and pH in seawater from these tests are shown in Appendix C-8.

Mysid Shrimp Entire Life-cycle Test

Mysid shrimp exposed to acephate in the 28-day test were adversely affected. At Day 7, mortality was significantly greater in an average measured concentration greater than 4,200 μg/ℓ than in the control (Table 6). At Day 14 and throughout the remainder of the exposure, mortality was significantly greater in a concentration of 1,400 μg/ℓ than in the control. After 28 days, mortality of mysids exposed to a concentration of 580 μg/ℓ was not significantly greater than mortality of the control mysids (Table 6).

There appeared to be a difference in time to brood pouch formation between mysids exposed to 4,200 μg/ℓ and the control. Brood pouches were first observed on Day 17 in control mysids, but on Day 19 in mysids exposed to 4,200 μg/ℓ. Time to brood pouch formation in mysids exposed to concentrations ≤2,000 μg/ℓ was similar to that of the control (Table 6).

The average number of offspring produced per female mysid exposed to measured concentrations of 2,000 and 4,200 μg/ℓ was significantly less than that produced by control mysids: 4.9 for mysids exposed to 2,000 μg/ℓ, 3.4 for mysids exposed to 4,200 μg/ℓ and 8.4 for the control (Table 7).

Survival of progeny of acephate-exposed mysids was not affected; none died in any treatment.

The maximum acceptable toxicant concentration (MATC, highest concentration that produced no significant effect and the lowest concentration in which significant effects were observed) of acephate, based on measured concentrations, was >580 <1,400 μg/ℓ and the application factor limits (MATC divided by the 96-h LC50 in a flow-through test) were 0.079 to 0.19.

Discussion

The acute toxicity of acephate is the lowest of the eight pesticides tested, and is characterized either by high EC50 or LC50 values or by no observed effect at concentrations tested (Table 4-a, 5-a, and 8). In static tests, acute intoxication in fish is characterized by signs of cholinergic poisoning (erratic swimming, loss of equilibrium, no reaction to gentle prodding, scoliosis, and rapid ventilation).

Other investigators have also noted that acephate is relatively non-toxic. Klaverkamp et al. (1975) in acute lethality studies on *Salmo gairdneri* with acephate at 15°C obtained 24-h LC50s of 895,000 μg/ℓ and 1,050,000 μg/ℓ. Miyaska and Kobayashi (1974) reported the 48-h TLM to "young carp" to be 30,000 μg/ℓ and to "shrimp," 299,000 μg/ℓ. Duangsawaski (1977) found that acephate directly inhibited cholinesterase activity in adult rainbow trout (*S. gairdneri*) and noted a decreased heart rate and increased ventilation rate, which indicate that the cardiovascular and respiratory systems of fish are important sites for acephate toxicity. Lyons et al. (1976) reported the 24-h LC50 of Orthene® (acephate) to frog (*Rana clamitans*) larvae exposed in static bioassays to be 6,433,000 μg/ℓ.

After a helicopter spraying of acephate on a Maine spruce-fir forest, Rabeni and Stanley (1979) studied stream biota from two ecosystems, one within and one outside the sprayed area. Post-spray residues of acephate peaked (140 μg/ℓ) within an hour after application, and disappeared at about 40% per day. No mortalities of stream biota were detected. However, acephate caused several perturbations to the stream ecosystem; drift of benthos temporarily increased; invertebrate standing crop remained unchanged; no dead fish were observed; brain acetylcholinesterase activity was depressed in suckers, but not in salmon or trout; brook trout altered their diet but their growth was unchanged. Evidence from this study indicate that acephate caused less impact on a forest stream ecosystem than other spruce budworm insecticides.

USDI (1979) described intensive management of coniferous forests where pesticides are used against spruce budworm epidemics, noting that potential contaminant problems exist for salmonid populations of the western and northeastern states. Orthene^R (acephate) was considered the most acceptable of five insecticides studied, but is not extensively used due to limited availability and high cost.

Available data indicate that acephate may be the least toxic of the insecticides tested. Acute lethality is not the manifestation of concern. LC50s and EC50s were high for all species in both static and flow-through tests. In the chronic toxicity test, there was little difference between concentrations that produced chronic effects on mysid shrimp and the 96-h LC50 to this invertebrate.

ALDICARB

Results

Acute Static Toxicity Tests

Algae--The 96-h EC50 (the calculated concentration that would inhibit growth by 50% in relation to control cultures) for Skeletonema costatum was greater than the highest concentration tested, 50,000 μg/ℓ (Table 4-a; Appendix C-1).

Invertebrates--The amount of aldicarb necessary to cause a 50% reduction in the number of normal oyster embryos in 48 h (48-h EC50) was 8,800 μg/ℓ (Table 4-a; Appendix D-1a and Appendix D-1b). The percentage reduction of embryos that developed normally to the straight-hinged, veliger stage after 48 h was from 9% in 1000 μg/ℓ to 71% in 32,000 μg/ℓ. Aldicarb was most toxic to the two crustacean species tested (Table 4-a). Mysid shrimp (Mysidopsis bahia) showed sensitivity at 13 μg/ℓ (10-15) (Appendix D-2), whereas white shrimp postlarvae (Penaeus stylirostris) had a 96-h LC50 of 72 μg/ℓ (65-82) (Appendix D-3).

Fishes--The 96-h LC50 of aldicarb to sheepshead minnows (*Cyprinodon variegatus*) was 168 µg/ℓ and to spot (*Leiostomus xanthurus*) 202 µg/ℓ (Table 4-a; Appendix D-4 and Appendix D-5).

Acute Flow-through Toxicity Test

Invertebrates--The 96-h LC50 of aldicarb to mysid shrimp (*Mysidopsis bahia*), based on measured concentrations, was 16 µg/ℓ (13-20) (Table 5-a and Appendix D-6). The 96-h LC50 of aldicarb to pink shrimp (*Penaeus duorarum*) based on measured concentrations, was 12 µg/ℓ (7.5-18) (Table 5-a; Appendix D-7). Ranges of DO and pH measured in seawater used in these tests are given in Appendix C-8.

Fishes--The 96-h LC50 of aldicarb for sheepshead minnows (*Cyprinodon variegatus*) was 41 µg/ℓ (55-72) (Table 5-a and Appendix D-8) and for pinfish (*Lagodon rhomboides*), 80 µg/ℓ (43-150) (Table 5-a and Appendix D-9). Ranges of DO and pH measured in seawater used in these tests are in Appendix C-8.

Mysid Shrimp Entire Life-cycle Test

Mysid shrimp exposed to aldicarb in the 28-day test were adversely affected by concentrations significantly less than the 96-h LC50 (Tables 5-a and 9). Significant mortality of parental generation shrimp occurred after 14 days of exposure to measured test concentrations of 1.5 and 2.1 µg/ℓ. The highest test concentration, 2.1 µg/ℓ, had an average 40% mortality by the end of test. There appeared to be a meaningful difference in time to brood pouch formation between mysids exposed to 2.1 µg/ℓ and the controls (Table 9). Brood pouches were first observed on Day 16 in the two controls and on Day 20 in mysids exposed to 2.1 µg/ℓ. No significant difference existed between the average number of offspring of mysids exposed to test concentration of 1.5 µg/ℓ and the average number of offspring produced by control mysids. No offspring were produced by mysids exposed to 2.1 µg/ℓ (Table 10). Survival of juveniles (F_1 generation) in a post-hatch exposure was not significantly affected after 4 to 8 days in either controls or any exposure concentration. The MATC (maximum acceptable toxicant concentration) based on measured concentrations was 1.0 to 1.5 µg/ℓ, and the application factor limits were 0.063-0.094.

Sheepshead Minnow Embryo/Juvenile Test

Hatching success of sheepshead minnows was not affected by exposure to ≤88 µg aldicarb/ℓ. Percentage survival from fertilization through embryonic development to hatching ranged from 91% to 98% in all exposure aquaria. Embryonic abnormalities or hatching delays were not observed.

A 28-day posthatch exposure to ≤88 µg aldicarb/ℓ did not significantly affect juvenile survival (Table 11), based on counts of living fish on Days 7, 14, 22, and 28. The majority of the mortality in all test concentrations occurred by Day 14 posthatch. No physical abnormalities were observed among juvenile fish in any treatment.

Growth (mean standard length) of juvenile fish was affected at the highest exposure concentration, 88 µg/ℓ (Table 12). Chemical analyses of fish that survived each test exposure did not yield any residues of aldicarb. The estimated MATC for embryos and juveniles of sheepshead minnows exposed to aldicarb was >50 <88 µg/ℓ. The 96-h LC50 (44 µg/ℓ; Table 5-a) was lower than the MATC limits. No obvious explanation for a lower LC50 value is evident. Bionomics Marine Research Laboratory conducted both tests, and the measured aldicarb concentrations in water from each test were approximately 60% of nominal and other critical test conditions (i.e., DO, temperature, etc.) were similar.

An attempt was made by personnel at Bionomics Marine Research Laboratory to conduct a life-cycle toxicity test with aldicarb and the grass shrimp, Palaemonetes pugio. Poor water quality and rapid growth of bacterial slime and algae in test tanks contributed to high mortality in the solvent control and several of the test concentrations. For these reasons, data from the test are not included here.

Discussion

Acute toxicity testing of aldicarb, in either static or flow-through 96-h exposures, showed it to be more acutely toxic to invertebrates than fish. The range of toxicity varied from 12 µg/ℓ to 72 µg/ℓ for marine invertebrates; and from 41 µg/ℓ to 202 µg/ℓ for marine fish (Tables 4-a and 5-a). Freshwater fish have similar or less susceptibility to aldicarb than estuarine species. Several investigators found the toxicity to bluegill (Lepomis macrochirus) to range from 76 to 100 µg/ℓ and that of rainbow trout to range from 560 to 880 µg/ℓ (Midwest Research Institute, EPA-540/1-75-013, 1975). A saltwater phytoplankter (Skeletonema costatum) and oyster larvae (Crassostrea virginica) were much less sensitive than either estuarine fish or invertebrate species tested (Table 4-a).

Longer-term life-cycle exposures of estuarine fish and invertebrate species to aldicarb once again revealed that invertebrate species are more sensitive. Results of a life-cycle toxicity test with mysid shrimp (Mysidopsis bahia) showed long-term effects to occur at significantly lower concentrations (1.5 and 2.1 µg/ℓ) than that at which acute toxicity occurred (16 µg/ℓ). Mortality of animals exposed to aldicarb concentrations $\geq$ 1.5 µg/ℓ was significantly greater than that of controls, but the difference did not become significant until Day 14 of the exposure. At the highest concentration tested, 2.1 µg/ℓ, there were also effects on reproduction (no release of young), as well as reduced survival.

CARBOPHENOTHION

Results

Acute Static Toxicity Tests

Algae--The 96-h EC50 for the marine diatom (*Skeletonema costatum*) exposed to carbophenothion was the lowest among the eight pesticides tested (Table 4-a, Appendix C-1). The 96-h EC50 and 95% confidence interval was 109 (100-115) µg carbophenothion/ℓ. The no-effect concentration was 10 µg/ℓ.

Invertebrates--The 48-h EC50 and 95% confidence intervals for embryos of the eastern oyster (*Crassostrea virginica*) exposed to carbophenothion were 99 (96-102) µg/ℓ (Table 4-a, Appendix E-1a, and Appendix E-1b). Stock solutions chemically analyzed from this test were 109% of nominal.

The 96-h LC50s and 95% confidence intervals for *Mysidopsis bahia* and *Penaeus stylirostris* are shown in Table 4-a, Appendix E-2, and Appendix E-3. These results were 13 (10-17) µg/ℓ for *M. bahia* and 4.3 (3.8-5.1) µg/ℓ for *P. stylirostris*.

Fishes--Sheepshead minnows (*Cyprinodon variegatus*) and spot (*Leiostomus xanthurus*) had strikingly different static toxicity test results when exposed to carbophenothion (Table 4-a, Appendix E-4 and Appendix E-5). The 96-h LC50s and 95% confidence intervals were 17 (14-21) µg/ℓ for *C. variegatus* and 500 (390-630) µg/ℓ for *L. xanthurus*. Thus *C. variegatus* was 29 times more sensitive; a very uncharacteristic difference as spot is typically more sensitive.

Acute Flow-through Toxicity Tests

Invertebrates--The 96-h LC50 values and 95% confidence intervals for the invertebrate species are listed in Table 5-a, and Appendices E-6 to E-10. Pink shrimp (*Penaeus duorarum*) was the most sensitive species (96-h LC50 = 0.47 µg/ℓ, 0.36-0.66) followed by *M. bahia* (3.0, 2.4-3.7) and wild *Palaemonetes pugio* (4.6, 3.8-6.2). Three acute studies were conducted on separate groups of *P. pugio*. Two groups consisted of animals that were hatched in the laboratory; the third was obtained from field collections. The LC50 values from three groups were not appreciably different, ranging from 2.0 to 4.6 µg/ℓ.

Fishes--Four fish species were exposed to carbophenothion in separate flow-through toxicity tests. Results of these studies (Table 5-a and Appendices E-11 to E-14) show a wide range in species sensitivity. Sheepshead minnows were the most sensitive with a 96-h LC50 of 2.8 (0.85-9.6) µg/ℓ; *Menidia menidia* (5.7-12) and pinfish (*Lagodon rhomboides*, 4.6-12) had identical LC50 values of 7.7 µg/ℓ, while spot (*Leiostomus xanthurus*) was relatively insensitive (96-h LC50 >206 µg/ℓ).

Mysid Shrimp Entire Life-cycle Test

Effects of carbophenothion on mysid shrimp (Mysidopsis bahia) in a 28-day life-cycle toxicity test were observed at concentrations relatively close to the measured 96-h LC50 of 3.0 µg/ℓ (Table 13). Mortality of adult mysids exposed to 0.3 and 4.1 µg/ℓ was significantly greater than mortality of controls. Mortality in the 0.30 µg/ℓ concentration was probably related to bacterial and fungal populations enhanced by the presence of triethylene glycol (TEG) as a solvent carrier and not to the presence of carbophenothion. This is further supported by the lack of significant mortality in the next three higher concentrations. Mortalities in 4.1 µg/ℓ were probably carbophenothion-related because onset of poisoning was early and paralleled effects seen in progeny.

Growth of parental mysid shrimp was significantly diminished in concentrations of carbophenothion 1.2 µg/ℓ and greater (Table 16). To obtain more consistent measurements from photographs, lengths were taken from the bottom of the cornea of the eyestalk to the statocyst at the base of the telson. Therefore, lengths may appear shorter than is normal for adult mysid shrimp.

Results of the reproductive portion of the test suggest effects at 4.1 µg/ℓ (Table 14). The total number of offspring, the number of offspring per female, and the number of days until onset of spawning, while not statistically different, appeared impacted in this concentration but not in any others.

Survival of juveniles for 10 days post release was significantly reduced in 4.1 µg/ℓ (Table 15). Behavioral aberrations, including abnormal and erratic swimming, were noted among juveniles reared in 1.9 and 4.1 µg/ℓ.

The maximum acceptable toxicant concentration (MATC) of carbophenothion for this mysid shrimp was >0.48 <1.2 µg/ℓ, based on statistically significant reduction in lengths. The measured 96-h LC50 for this animal is 3.0 µg carbophenothion/ℓ. The application factor limits then are >0.16 <0.4.

Grass Shrimp Entire Life-cycle Test

Survival of adult shrimp in 2.9 µg/ℓ, the highest concentration, was significantly less than controls (Table 17). Survival of shrimp on Day 33 was 24% in 2.9 µg/ℓ and 91 to 98% in all other treatments. Survival at the end of the test was 45 to 75% for all treatments, except 2.9 µg/ℓ, where none remained.

The spawning of grass shrimp was significantly affected by carbophenothion (Table 18 and Figure 1). Ovarian maturation and onset of spawning, as measured by deposition of eggs on pleopods, first occurred on Day 32 in 2.9 µg/ℓ-exposed shrimp. In contrast, it was first observed on Days 16 and 18 in controls and Days 14 to 18 in all other treatments. The total number of spawning females was significantly reduced compared to controls at concentrations of 0.36 μg/ℓ and above.

Carbophenothion did not significantly affect the number of eggs produced/female or the number of larvae to hatch in any exposure concentration (Table 18). While egg counts were not made in the highest concentration due to insufficient availability, it did not appear that egg numbers were diminished. This is supported by the fact that the number of hatched larvae was not affected in this concentration.

Repeated attempts to rear larvae under conditions of continuing toxicant exposure were only partially successful due to salinity fluctuations and other test complications (Appendix E-15). Therefore, effects on larval survival were only noted for the first 10 days of larval development.

Carbophenothion significantly diminished survival of larvae reared in 2.9 μg/ℓ, as compared to those reared in the controls and all other test concentrations. Survival of larvae reared in 2.9 μg/ℓ averaged 5%, while in all other treatments it averaged 82 to 90% on Day 10 (Table 19). Larvae hatched from five ovigerous females exposed to 2.9 μg/ℓ were reared separately. Complete mortality occurred within 3 to 8 days in larvae hatched from three of the females and only 5% and 26% of the larvae from the other two females survived to Day 10. All larvae hatched in 2.9 μg/ℓ were hyperactive, swam in a chaotic manner, and their response to light was abnormal. Although larvae hatched in 1.3 μg/ℓ also demonstrated a disrupted response to light, survival was not impaired.

Growth of shrimp was not significantly affected by carbophenothion (Table 20). Weight measurements of all survivors of the 249-day exposure did not reveal any differences between control and exposed shrimp.

The average bioconcentration factor of carbophenothion in shrimp tissue was low: 420X that measured in water (Table 21). Carbophenothion was only detected in tissues of shrimp exposed to ≥0.36 μg/ℓ in water. It was these concentrations in which reproductive effects, i.e., impaired spawning response, were noticed (Figure 1, Table 18).

The maximum acceptable toxicant concentration (MATC) for grass shrimp, based on impaired reproduction, was >0.22 μg/ℓ <0.36 μg/ℓ and the 96-h LC50 was 2.9 (2.5 to 3.3) μg/ℓ. The application factor limits then are >0.076 <0.12.

Sheepshead Minnow Embryo/Juvenile Test

Continuous exposure of sheepshead minnow embryos to carbophenothion caused no significant difference ($\alpha = 0.05$) in the percentage that hatched (Table 22). Survival of embryos to hatching ranged from 85 to 95% per treatment.

Carbophenothion was lethal to hatched fish at concentrations of 11 and 5.4 μg/ℓ (Table 22). All fish exposed to 11 μg/ℓ died by Day 18 post-fertilization and 96% of those exposed to 5.4 μg/ℓ died within 28 days. Although survival of embryos or hatched fish was not significantly affected ($\alpha = 0.05$) by exposure to 2.8 μg carbophenothion/ℓ, the cumulative effect was significant with only 79% survival through 28 days (Table 22).

The occurrence, frequency, and severity of signs of carbophenothion poisoning were dependent upon the exposure concentration. Fish exposed to 0.36 and 0.59 μg/ℓ appeared unaffected. Although occasional abnormal lateral flexure of the body was observed in fish exposed to 1.3 μg/ℓ during the first week after hatching, these fish appeared unaffected during the remainder of the experiment. Signs of poisoning observed in fish exposed to 2.8 μg/ℓ were the occasional occurrence of darkened areas on the posterior one-third of the body, lethargy, abnormal lateral flexure of the body, and an abnormally thickened appearance when the fish were observed from a dorsal or ventral view. Signs of poisoning observed in fish exposed to 5.4 and 11 μg/ℓ were similar to those observed in the 2.8 μg/ℓ exposure, except they did not exhibit abnormal discoloration. However, muscular tetany was frequently observed in these fish when the egg cups were placed on a lighted stage for observation.

Although more food was available per fish in 2.8 μg carbophenothion/ℓ because of significant mortality, these fish were significantly shorter in standard length ($\alpha = 0.05$) than those in lower concentrations and in the control (Table 23). Fish exposed to 5.4 μg/ℓ averaged 9.7 mm standard length, but were excluded from the length analysis because there were so few survivors and there was much more available food per fish than in other treatments.

The results of this study imply that the estimated MATC for carbophenothion is between 1.3 and 2.8 μg/ℓ.

Carbophenothion accumulated in the tissues of fish surviving the 28-day experiment (Table 23). In the three concentrations for which residues were determined, the BCF averaged 950 (range 780 to 1,200).

Pathological findings suggest a threshold of effect between 1.3 and 2.8 μg carbophenothion/ℓ. Over 60% of those fish examined from 2.8 and 5.4 μg/ℓ had vertebral dysplasia consisting of abnormal thickening (5 to 15X normal) of vertebral walls (Fig. 2), particularly in the caudal area and occasionally in the trunk region. Foci or clusters of osteoblast-like cells were associated with the dysplasia, and osteocytes were included within the thickened bone. Fish exposed to carbophenothion concentrations <1.3 µg/ℓ were pathologically indistinguishable from control animals. This vertebral dysplasia is similar to, but less pronounced than, the dysplasia reported by Couch et al. (1979) for sheepshead minnows exposed to 5.5 µg/ℓ of the herbicide trifluralin.

Benthic Animal Community Test

Concentrations (nominal) of 0.01, 0.1, and 1.0 µg carbophenothion/ℓ had no significant adverse effects on the total numbers of individuals and species of macrobenthic animals colonizing sand in aquaria (Table 24). A total of 1,600 animals representing 41 species of 7 phyla were collected from control aquaria and from those exposed for 8 weeks, 3 days to carbophenothion. Numerically dominant phyla were Mollusca, Arthropoda, and Annelida (Table 25). Averages and ranges of measured concentrations of carbophenothion were: Control = nondetectable (limit of detection was 0.05 µg/ℓ); 0.01 µg/ℓ nominal = nondetectable; 0.1 µg/ℓ nominal = 0.1 (0.06-0.25) µg/ℓ; and 1.0 µg/ℓ nominal = 0.9 (0.52- 1.3) µg/ℓ. Seawater salinity averaged 20 °/oo (5.5- 31.5 °/oo) and temperature 19°C (15.0-23.0°C).

After eight weeks and three days exposure, the clam (*Mulinia lateralis*) bioconcentrated carbophenothion to 5,100 times that measured in exposure water.

Bioconcentration Test

Spot exposed to 2.9 µg/ℓ (5.0 µg/ℓ, nominal concentration) carbophenothion bioconcentrated the insecticide rapidly, reaching 90% of steady state in approximately 3 days (Figure 3). Uptake data for the 36 µg/ℓ (50 µg/ℓ, nominal concentration) were not illustrated because of excessive mortality. Mortality was not expected in this test because the acute toxicity of carbophenothion to spot was not great (96-h LC50 >206 µg/ℓ; Table 5-a). At steady state, the concentration of carbophenothion in whole spot exposed to 2.9 µg/ℓ was 1.8 µg/g, a bioconcentration factor of 620. In comparison, spot surviving a 96-h carbophenothion exposure bioconcentrated an average of 140 X. Reasons for this difference are unclear; however, the elevated concentrations of carbophenothion in the water of the acute study (>135 µg/ℓ) may have altered the rate of carbophenothion uptake.

After four days in carbophenothion-free seawater, only 10% of the steady-state concentration (0.18 µg/g) was measured in spot tissues; no fish were available beyond Day 4.

Persistence

The Log P for carbophenothion is 4.9 (Table 26). If this value is applied to the Log P/Log BCF regression curve of Veith (1979a), the BCF for fathead minnows (Pimephales promelas) is approximately 2900X. In a 28-day BCF study at steady state, spot (Leiostomus xanthurus) concentrated carbophenothion 620X.

Persistence tests on carbophenothion show it to be the most persistent of the pesticides tested (Table 27). Untreated and sterile sediment/water systems retained 57% after 5 days and 61% after 7 days, respectively, suggesting that biological processes are not responsible for the degradation of carbophenothion. This is contrary to studies with the other pesticides. Carbophenothion was not detected in the resin traps of any sediment/water systems, but in the seawater systems all of the pesticide that was accounted for (41%) was present in the resin trap. Presumably, hydrolysis in the seawater systems removed over half of the pesticide and sorption to sediments in the sediment/water system interferred with this degradative process and resulted in increased persistence. The observations that carbophenothion was purgeable and adsorbed to sediment predict a low potential for persistence in the water of aquatic systems. The sediment compartment remains the key factor in the ecological implications of carbophenothion in the aquatic environment.

Discusssion

The acute toxicity of carbophenothion to species of marine organisms we tested is similar to that reported for freshwater species. The 96-h LC50 of the insecticide to the glass shrimp (Palaemonetes kadiakensis) was 1.2 µg/ℓ (Sanders 1972); for various ages of the estuarine shrimp, P. pugio, the LC50 was between 2.0 and 4.6 µg/ℓ (Table 5-a). Sanders (1969) reported that the 96-h LC50 for the scud (Gammarus lacustris) was 5.2 µg/ℓ. The LC50 or EC50 values we report for invertebrate species range from 99 µg/ℓ for the larvae of the eastern oyster to 4.3 µg/ℓ for Penaeus stylirostris in static tests (Table 4-a).

The BCF from the bioconcentration test was one-fourth of that calculated for a freshwater fish. Our steady-state BCF in the spot study was 620, that calculated for fathead minnows was 2900 (Veith, 1979b). Persistence tests indicate that carbophenothion degradation is not biologically mediated; hydrolysis may be responsible for some degradation of the compound. As in many compounds with chemical and physical properties similar to carbophenothion, the sediment compartment is likely to be the key to the fate of this insecticide in the aquatic environment.

Carbophenothion has been found at concentrations from 0.103 to 0.109 µg/g in juvenile estuarine fishes from Texas and Maryland (Butler and Schultzmann, 1978). Residues probably are associated with agricultural activities in those areas. Our bioconcentration and persistence data indicate that carbophenothion may not remain in water or tissues for long periods of time; we assume that the fish were recently exposed.

In summary, carbophenothion is acutely toxic to three invertebrate and fish species at concentrations <20 µg/ℓ. Chronic toxicity tests on *Mysidopsis bahia*, *Palaemonetes pugio*, and *Cyprinodon variegatus* demonstrate that concentrations ranging from 0.36 to 2.8 µg/ℓ produce adverse effects. Bioconcentration of carbophenothion by spot achieved 90% steady state in three days and depurated to 10% after four days; the steady-state BCF was 620. Persistence studies indicated minimal biological degradation, with the sediment compartment serving as a sink.

DEF

Results

Acute Static Toxicity Tests

Algae--The 96-h EC50 and 95% confidence interval for marine diatoms (*Skeletonema costatum*) exposed to DEF were 366 (315-391) µg/ℓ (Table 4-a, Appendix C-1); the no-effect concentration was 200 µg/ℓ.

Invertebrates--The 48-h EC50 for embryos of the eastern oyster (*Crassostrea virginica*) exposed to DEF was 197 (120-323) µg/ℓ (Table 4-a, and Appendix F-1a and Appendix F-1b).

Two crustaceans exposed to DEF were mysid shrimp (*Mysidopsis bahia*) and postlarval white shrimp (*Penaeus stylirostris*). Results of these acute static tests are shown in Table 4-a, Appendix F-2, and Appendix F-3: the 96-h LC50s and 95% confidence intervals were 5.8 (4.4-6.8) µg/ℓ for *M. bahia*, and 25 (16-49) µg/ℓ for *P. stylirostris*. Mysids were roughly 4X more sensitive to DEF than were postlarval penaeid shrimp.

Fishes--Sheepshead minnow (*Cyprinodon variegatus*) and spot (*Leiostomus xanthurus*) acute static DEF toxicity test results (Table 4-a, Appendix F-4, and Appendix F-5) expressed as the 96-h LC50 and 95% confidence interval were 440 (410-473) µg/ℓ and 158 (135-320) µg/ℓ, respectively. The DEF toxicity to *L. xanthurus* was approximately 3X greater than that for *C. variegatus*.

Acute Flow-through Toxicity Tests

Invertebrates--The 96-h LC50 of DEF to mysid shrimp, based on measured concentrations, was 4.6 (4.2-4.9) µg/ℓ (Table 5-a, Appendix F-6). The 96-h LC50 of DEF to pink shrimp (Penaeus duorarum) based on measured concentrations was 14 (10-18) µg/ℓ (Table 5-a and Appendix F-7); that for grass shrimp (Palaemonetes pugio) was 22 µg/ℓ (Appendix F-8).

Fishes--The 96-h LC50 of DEF to pinfish (Lagodon rhomboides) based on measured concentrations was 290 (240-379) µg/ℓ (Table 5-a and Appendix F-9); that for spot was 130 (100-170) µg/ℓ (Appendix F-10). Sheepshead minnows suffered no mortality after exposure to 440 µg DEF/ℓ (Table 5-a and Appendix F-11).

Mysid Shrimp Entire Life-cycle Test

In a life-cycle toxicity test (Tables 28 and 29) in flowing sea-water at ERL,GB, mysid shrimp (Mysidopsis bahia) were exposed to 0.34, 0.68, 1.7, and 3.3 µg DEF/ℓ (measured concentrations). No mysids survived the 28-day exposure to 3.3 µg/ℓ. A significant reduction in survival was observed in 0.68 and 1.7 µg DEF/ℓ at 28 days. No significant effect on survival occurred at 0.34 µg/ℓ.

Reproduction, expressed as the number of young produced per female, was significantly reduced (at least 50%) in all exposure concentrations. At 1.7 µg/ℓ, reproduction failed completely. Where reproduction was partially successful (0.34 and 0.68 µg/ℓ), the onset was delayed.

The MATC limits for DEF were not determined since the no-effect concentration must be less than 0.34 µg/ℓ. Therefore, the application factor is a value <0.074 of the 96-h LC50.

Persistence

The Log P for DEF is 5.7 (Table 26). If this value is applied to the Log P/Log BCF regression curve of Veith (1979a), the BCF for fathead minnows (Pimephales promelas) is approximately 14,000X. The pinfish (Lagodon rhomboides) concentrated DEF 350X in a 96-h experiment. This value is low because either steady state was not achieved in 96-h exposure or the compound was rapidly metabolized.

The results of persistence tests for DEF are in Table 30 and indicate that the pesticide disappeared most rapidly from the untreated sediment/water systems, with 20% remaining after 5 days. In similar studies with methyl parathion, only 3% of the pesticide was detected after 5 days. In contrast, the sterile DEF systems retained 77% of the pesticide after 7 days and demonstrated the importance of biological processes in the disappearance of the pesticide. While none of the systems mentioned above displayed any detectable pesticide loss in the air, the DEF seawater systems contained 12% of the original pesticide in the resin trap after 7 days. Similar to previous studies with EPN, only 38% of DEF was recovered from the water of the seawater systems after 7 days, with the remaining 50% loss attributed to hydrolysis in the water compartment. As in EPN studies, sorption of DEF to sediments in the sterile systems reduced hydrolysis either because the pesticide was not susceptible to hydrolytic processes in a sorbed state or because biological processes were responsible for hydrolysis in the water.

Discussion

The acute toxicity of DEF, a systemic herbicide widely used to defoliate cotton and for the control of undesirable plant species, to aquatic organisms is most pronounced in crustaceans. The static and flowing acute results for *M. bahia* (Tables 4-a and 5-a) are not statistically different. Also, our *Penaeus* sp. static and flowing 96-h LC50s (Tables 4-a and 5-a) are both within 2X of an earlier 48-h LC50 by Lowe (personal communication: 1980, Jack I. Lowe, U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida). He determined that the 96-h EC50 (the concentration of DEF causing a 50% decrease in shell growth) in eastern oysters (*Crassostrea virginica*) was 100 µg/ℓ. The 48-h LC50 for brown shrimp (*Penaeus aztecus*) was 28 µg/ℓ; and the 48-h LC50 for spot (*Leiostomus xanthurus*) was 240 µg/ℓ. Sanders (1969) determined the acute static toxicity of DEF to freshwater scuds (*Gammarus lacustris*). The 96-h LC50 and 95% confidence interval was 100 (78-150) µg/ℓ.

Our toxicity data indicate that fishes are less sensitive to DEF than crustaceans. The static 96-h LC50s for *C. variegatus* and *L. xanthurus* were 440 (410-473) µg/ℓ and 158 (135-320) µg/ℓ. Flow-through acute test results for *L. rhomboides*, *L. xanthurus*, and *C. variegatus* were 130 (100-170) µg/ℓ, 290 (240-379) µg/ℓ, and >440 µg/ℓ, respectively.

Fabacher et al. (1976) reported a pronounced increase in the toxicity of DEF by methyl parathion in mosquitofish (*Gambusia affinis*). The 24-h LC50 of DEF alone to *G. affinis* is approximately 800 µg/ℓ. No mortality occurred within 24 h in *G. affinis* treated with 500 µg DEF/ℓ. In *G. affinis* treated with 5,000 µg methyl parathion/ℓ alone, mortality was 8%. However, mortality was 89% in fish treated with 500 µg DEF/ℓ combined with 5,000 µg methyl parathion/ℓ.

Caldwell et al. (1979) conducted toxicity tests with DEF on several life stages of the Dungeness crab (*Cancer magister*). The percentage of hatched crab prezoeae successfully molting to the first zoeal stage during the 24-h test period was affected at 100 µg DEF/ℓ. Motility of developing first stage zoeae declined. At 10 µg/ℓ 80% were motile, and motility decreased to below 40% at 33 and 100 µg/ℓ after 24-h exposure. Zoeae exposed to the high concentration (6.9 µg/ℓ) molted one day later than controls through the first three instars. In long-term toxicity tests, zoeae survival exceeded 80% in controls and in each concentration (0.04, 0.95, and 6.9 µg/ℓ) of DEF until Day 50. Then survival decreased in all cultures until the test was terminated on Day 69. Chronically exposed larvae were killed at concentrations similar to those in acute toxicity tests. The lethal threshold for DEF is approximately 6.9 µg/ℓ. Survival of juvenile crabs was reduced at 270 µg DEF/ℓ, but not at lower concentrations. Adult crabs chronically exposed to DEF exhibited reduced survival at 380 and 2600 µg/ℓ compared to controls. Survival of adult crabs was significantly reduced after 50 days at 380 µg/ℓ and 32 days at 2,600 µg/ℓ. The no-effect concentration for developing *C. magister* exposed to DEF was estimated to lie between 0.95 and 6.9 µg/ℓ.

In our life-cycle toxicity test with mysids, the lower value for the MATC limits was not determined for *M. bahia* exposed to DEF since a no-effect concentration was not observed. Therefore, the MATC for reproductive effects is less than the lowest concentration, 0.34 µg/ℓ. The application factor is less than 0.074 (based on a chronic MATC of <0.34 µg/ℓ and a measured acute flow-through 96-h LC50 of 4.6 µg/ℓ), which indicates a chronic exposure hazard at least an order of magnitude greater than the acute toxicity.

Evaluation of toxicity and fate data is necessary to predict the hazard of DEF to marine organisms. Persistence tests indicate that DEF degrades rapidly in the presence of estuarine sediments. Application of DEF near coastal areas or watersheds could affect estuarine crustaceans, even at exposure concentrations below 1 µg/ℓ.

EPN

Results

Acute Static Toxicity Tests

Algae--The 96-h EC50 for *Skeletonema costatum* exposed to EPN was 340 µg/ℓ (Table 4-b and Appendix C-1), the second lowest of the eight insecticides tested.

Invertebrates--The 48-h EC50 of 2,200 µg/ℓ to eastern oysters (*Crassostrea virginica*) (Table 4-b, Appendix G-1a and Appendix G-1b) was over 15 times greater than that for sheepshead minnows (*Cyprinodon variegatus*), the next least sensitive species. Reduction in numbers of oyster embryos that developed normally to the straight-hinged, veliger stage after 48 h ranged from 4% in 320 µg/ℓ to 76% in 5,600 µg/ℓ (Appendix G-1b).

Acute toxicity of EPN to crustaceans (*Mysidopsis bahia* and *Penaeus stylirostris*) is shown in Table 4-b, Appendix G-2, and

Appendix G-3. The 96-h LC50 and 95% confidence intervals for *M. bahia* were 13 µg/ℓ (10 to 18); for *P. stylirostris*, 4.6 µg/ℓ (2.4-9.0).

Fishes--The 96-h LC50 values and 95% confidence intervals for the two fish species exposed to EPN differed greatly (Table 4-b, Appendix G-4, and Appendix G-5). Spot (*Leiostomus xanthurus*) were more sensitive, LC50 = 37 µg/ℓ (33 to 40), than sheepshead minnows, 140 µg/ℓ (59 to 500).

Acute Flow-through Toxicity Tests

Invertebrates--The crustaceans, *M. bahia* and *P. duorarum*, were particularly sensitive to EPN in flow-through tests (Table 5-b, Appendix G-6, Appendix G-7). The 96-h LC50 and 95% confidence limits for *M. bahia* were 3.4 µg/ℓ (2.5 to 5.8 µg/ℓ), a value nearly one-fourth that measured in static tests (Table 4-b). *P. duorarum* were more sensitive to EPN (96-h LC50 = 0.29 µg/ℓ).

Fishes--the 96-h LC50 values for the three fish species exposed to EPN are listed in Table 5-b, Appendix G-8, Appendix G-9, and Appendix G-10. Pinfish (*L. rhomboides*) was the most sensitive fish species with LC50 and 95% confidence limits being 18 µg/ℓ (15 to 24 µg/ℓ); spot, 26 µg/ℓ (19 to 34 µg/ℓ), and sheepshead minnows, 190 µg/ℓ (150 to 260 µg/ℓ).

Mysid Shrimp Entire Life-cycle Tests

The lowest concentration of EPN that produced no adverse effect on survival or fecundity was 0.44 µg/ℓ (Tables 31 and 32). A concentration of 4.1 µg EPN/ℓ adversely affected both survival and number of young produced per female (Table 32). Approximately 63% of the deaths in 4.1 µg/ℓ occurred during the final 14 days of the test and may have been a result of the increased stress associated with reproduction and accumulated stress of the toxicant. The number of young produced was less in 4.1 µg/ℓ than in the control aquaria; and in most chambers, no young were produced. Of nine females in 4.1 µg EPN/ℓ, only two produced young during the test. This could have been a result of toxicant-induced sterility in either male or female mysids or of disruption of their sexual behavior.

The MATC for EPN is >0.44 <3.4 µg/ℓ; the 96-h LC50 in a flow-through test was 3.4 µg/ℓ. Therefore, the application factor was 0.13 to 1.0 of the 96-h LC50. Because the limits on the MATC were about an order of magnitude apart, the estimated "safe" concentration is poorly defined.

Sheepshead Minnow Entire Life-cycle Tests

Survival and signs of poisoning in parental fish--Survival of embryos to hatching and of hatched fish to Day 28 in the six EPN concentrations was not significantly different from that of controls, but significantly fewer fish survived from Day 28 to Day 140 in 7.9 µg/ℓ (Table 33). Survival of embryos to hatching averaged 88% (range, 83 to 94% per concentration) and survival of fish from hatching through Day 28 averaged 97% (range, 96 to 99% per concentration). Survival from Day 28 to 140 was significantly less in fish exposed to 7.9 µg/ℓ than in controls. Overall survival of embryos to Day 140, calculated from data on embryo, juvenile, and adult survival, appeared to be concentration-dependent. Overall survival averaged 87% (range, 82 to 90%) for control fish and fish exposed to 0.25, 0.50, and 0.88 µg/ℓ. Survival decreased from 73 to 67% as concentration increased from 2.2 to 7.9 µg/ℓ. Survival of fish from Day 140 to the end of the test (Day 265) could not be analyzed because of mortality from excessive handling or mortality associated with spawning trials.

External signs of poisoning observed in fish exposed to 2.2 µg/ℓ through Day 28 were lethargy and edema. Fish exposed to 4.1 and 7.9 µg/ℓ exhibited lethargy, edema, and abnormal lateral flexure of the body.

Signs of poisoning observed in juvenile and adult fish after release from the egg cups were: occasional blackening of the posterior third of the body of fish exposed to 0.25, 0.50, and 0.88 µg/ℓ; blackening plus reduced feeding, abnormal lateral flexure of the body, and abnormal muscular tetany in fish exposed to 2.2, 4.1, and 7.9 µg/ℓ. Fish exposed to 4.1 and 7.9 µg/ℓ were lethargic. Fish exposed to 2.2, 4.1, and 7.9 µg/ℓ frequently experienced muscular tetany when food was first placed in the aquaria. Generally, the frequency of occurrence of these signs seemed dependent on the exposure concentration.

Growth of parental fish--Chronic exposure of sheepshead minnows to EPN affected their growth (Table 34). Average standard lengths of juvenile control fish at Day 28 were greater than lengths of fish in each concentration tested. Thereafter, fish exposed to 7.9 µg/ℓ were always significantly shorter than control fish. Their lengths averaged 2.6 to 3.9 mm less than lengths of control fish between Days 60 and 140, (11 to 16% shorter than controls).

We believe that the significant decrease in lengths of fish exposed to 7.9 µg/ℓ may be biologically significant, but that significant decreases in lengths of fish in lower concentrations observed at Day 28 and, in one instance, at Day 60 are probably not biologically significant. Differences in lengths in these lower concentrations at Day 28 and 60 are within measurement error of about ± 1 mm and are not consistent throughout this experiment; thus, we doubt their biological significance.

Reproduction--Egg production was extremely variable among the three different spawning trials and between duplicates within the same trials (Table 35). Although control fish averaged 7.9 eggs per female per day and fish in 7.9 µg EPN/ℓ averaged only 0.5 eggs per female per day, the variability was so large that significant differences (α = 0.05) were not observed.

Percentage fertility averaged ≥97.9% in all concentrations, except 7.9 µg/ℓ, in which fertility averaged 93.8% and was significantly different from all other concentrations. However, statistical analyses of fertility data revealed no differences between controls and fish exposed during individual spawning trials.

When our experiment is compared with published accounts of the effects of toxicants on sheepshead minnow reproduction in which data are presented by spawning groups (Parrish et al., 1977; Parrish et al., 1978; Goodman et al., 1979), this EPN test is the only experiment in which there were control groups that did not spawn. The average number of eggs per female per day, for a single spawning group (3 females and 2 males) in control or control-with-carrier aquaria or both, in these published studies ranged from four in a life-cycle toxicity test with chlordane (Parrish et al., 1978) and 12 to 39 in a life-cycle toxicity test with methoxychlor (Parrish et al., 1977).

Two difficulties were encountered in this experiment that probably contributed to the large variability in the reproduction data. First, it was unusually difficult to determine the sex of the fish when placing them in the spawning chambers. Sheepshead minnows are sexually dichromatic, but differences in coloration are sometimes subtle. At present, we do not know whether the lack of expression of sexual dichromatism was due to genetics of the brood stock or to some factor in our exposure system. Although we attempted to place 3 females and 2 males in each spawning group, the ratio was frequently wrong when determined by dissection after trials I and III. The sex of fish from spawning trial II was not determined by dissection because we were reasonably confident of the sex ratio and we needed to keep the fish alive for subsequent experiments. Data analyses were based on the average number of eggs per female per day, but there may have been differences in egg production attributable to the male/-female ratio. Secondly, we had difficulty in maintaining DO levels during the reproductive portion of the experiment (Table 36). Average DO concentrations were 2.5 mg/ℓ in trial I, 3.6 mg/ℓ in trial II, and 3.3 mg/ℓ in trial III.

Survival, growth, and signs of poisoning in progeny--There was no significant (α = 0.05) toxicant-related effect on survival of progeny from fish continuously exposed to average measured concentrations of 0.25, 0.50, and 0.88 µg EPN/ℓ. Survival of embryos to hatching averaged 88% (range, 81 to 92% per concentration); and of the fish that hatched, an average of 96% (range, 93 to 100% per concentration) survived through Day 28 (Table 37).

The average standard length of surviving progeny was unaffected by EPN exposure through 28 days (Table 37). Average standard lengths of progeny ranged from 8.6 mm in 1.25 μg/ℓ to 9.0 mm in 0.31 μg/ℓ; control fish averaged 8.9 mm.

No signs of poisoning were observed in progeny exposed to 0.25 and 0.50 μg EPN/ℓ. Progeny exposed to 0.88 μg/ℓ infrequently appeared to be affected; signs of poisoning were those described for parental fish.

MATC and application factor--The MATC for sheepshead minnows continuously exposed to EPN at 30°C, lies between 4.1 and 7.9 μg/ℓ based on: (1) significantly reduced survival from Day 28 to 140; (2) reduced length of fish; and (3) reduced fertility of eggs from fish exposed to 7.9 μg EPN/ℓ of water. However, Cripe (Appendix G-11) determined the critical swimming speed of adult fish during this exposure and found that the threshold level of effect on swimming performance lies between 0.88 and 2.2 μg/ℓ.

The application factor for sheepshead minnows, obtained by dividing the MATC limits based on reduced survival, growth, and egg fertility by the 96-h LC50 (190 μg/ℓ at 25°C; Table 5-b), lies between 0.022 and 0.042. Although we are unaware of any MATC being based on critical swimming speed, if the threshold levels reported by Cripe (Appendix G-11) were used to calculate the application factor, it would lie between 0.0046 and 0.012.

Bioconcentration--EPN was bioconcentrated by all life stages of the sheepshead minnow (Table 38). Concentrations in fish or their embryos generally increased with increasing exposure concentration. In embryos and 28-day juveniles, tissue concentrations were generally less for the same concentration of exposure than in adult fish. Concentrations in males and in females varied considerably, but seemed to be similar.

Bioconcentration factors (BCFs) appeared to be greater in adult fish than in juveniles or embryos (Table 39), averaging 1,000 for juvenile fish, 5,800 for adult males, 5,300 for adult females, 1,300 for embryos from exposed fish, and 650 for juvenile progeny from exposed parents. Bioconcentration factors in each of the three groups of adult fish appeared to increase with increasing concentration of exposure. Bioconcentration factors averaged 2,100 for adult fish exposed to the three lowest EPN concentrations and 7,700 for adult fish exposed to the three highest EPN concentrations. This increase in bioconcentration factor with increase in concentration of exposure is unusual. This anomaly may occur because fish in the highest concentrations were adversely affected by EPN, which may alter usual uptake and depuration rates in adult sheepshead minnows.

Acetylcholinesterase activity--Acetylcholinesterase (AChE) inhibition of parental fish from spawning groups I and III was directly dependent on the exposure concentration (Table 40). Average AChE activity throughout the study ranged from 38.0% inhibition in fish exposed to 0.25 µg/ℓ to 85.5% inhibition in fish exposed to 7.9 µg/ℓ, with good correlation in extent of inhibition between the two spawning groups.

Bioconcentration Tests

Pinfish exposed to 2.4 µg/ℓ bioconcentrated EPN rapidly, reaching an apparent steady state in approximately two days (Figure 4). Uptake and depuration data for the 0.17 µg/ℓ (0.25 µg/ℓ, nominal concentration) exposure were not illustrated, because residues in these tissues were usually below the level of detectability (0.02 µg/g). At steady state, the concentration of EPN in whole pinfish exposed to 2.4 µg/ℓ was approximately 1.7 µg/g, giving a bioconcentration factor of 700. In comparison, pinfish that survived a 96-h exposure bioconcentrated EPN an average of 740X. Additional species and their mean EPN bioconcentration factors were pink shrimp, <48 (none detectable), and spot, 260X.

After a four-day post-exposure period, only one pinfish of four sampled had detectable (0.04 µg/g) EPN in its tissues; after eight days, no EPN was detected in any fish sampled.

Persistence

The Log P for EPN is 4.0 (Table 26). If this value is applied to the Log P/Log BCF regression curve for fathead minnows (Pimephales promelas) of Veith (1979a), the BCF is approximately 500X. The BCF observed for pinfish exposed to EPN was 700X, which correlates well with the predicted value and demonstrates a relatively low potential for bioaccumulation of EPN in the tissues of aquatic organisms. In contrast, in a life-cycle test, sheepshead minnows bioconcentrated EPN 650 to 5,800X the water concentration, depending on life-stage.

In the results of persistence tests with EPN (Table 41), the untreated systems retained much greater amounts of pesticide after five days than did similar methyl parathion systems. Like methyl parathion systems, used here as a reference, sterile EPN systems showed less disappearance than untreated systems, with 79% of the starting concentration of the pesticide being recovered after seven days. More EPN disappeared from seawater systems than did methyl parathion after seven days, which indicates that EPN is more susceptible to hydrolysis in the water column than is methyl parathion. This observation is supported by the diminished disappearance of EPN when sorbed to sediments in the sterile systems. The untreated systems displayed the greater disappearance of EPN, due to the combination of hydrolysis in the water column and biological processes in the sediment compartments. Volatility was not a significant factor in these studies since EPN was not detected in the resin traps. Studies with natural sunlight were not conducted for EPN.

Discussion

The 96-h LC50s were similar for static and flow-through toxicity tests with EPN in which the same species were exposed (Table 4-b and 5-b). Differences between these values for the species tested (*M. bahia*, *C. variegatus*, and *L. xanthurus*) ranged from a factor of 1.3 to 3.8.

Our 96-h EPN acute toxicity test results are comparable to those for freshwater species. Sanders (1972) reported 96-h LC50 values of 6.8 μg/ℓ for scud (*Gammarus fasciatus*) and 0.56 μg/ℓ for glass shrimp (*Palaemonetes kadiakensis*). Pickering et al. (1962) reported the 96-h LC50 values of EPN for several freshwater fishes: bluegill (*Lepomis macrochirus*), 100 μg/ℓ; fathead minnows (*Pimephales promelas*), 255 μg/ℓ; goldfish (*Carassius auratus*), 450 μg/ℓ; and guppies (*Lebistes reticulatus*), 32 μg/ℓ. Korn and Earnest (1974) exposed juvenile striped bass (*Morone saxatilis*) to EPN in flowing seawater and calculated a 96-h LC50 of 60 μg/ℓ. Our LC50s for spot (25.6 μg/ℓ, flow-through tests; 36.6 μg/ℓ, static tests) and pinfish (18.3 μg/ℓ, flow-through tests) indicate that these species are more sensitive than freshwater fishes, whereas sensitivity of sheepshead minnows to EPN (LC50 = 189 μg/ℓ, flow-through tests; 144 μg/ℓ, static tests) was similar to that of fathead minnows and bluegills.

Pink shrimp (*P. duorarum*) were especially sensitive to EPN (LC50 = 0.29 μg/ℓ), and there was even a 20% mortality among shrimp exposed for 96 h to a nondetectable concentration. Further, since no EPN was detected in tissues of shrimp exposed to concentrations as great as 0.42 μg/ℓ, it is conceivable that pink shrimp in estuarine habitats could suffer significant mortality if exposed for more than 96 h to EPN concentrations below our chemical detection limits, and the identity of the toxicant that caused the mortality could not be determined by residue analysis.

The MATC for EPN in chronic studies with mysid shrimp lies between 0.44 and 3.4 μg/ℓ; that for sheepshead minnows, between 4.1 and 7.9 μg/ℓ (Table 8). The application factor for sheepshead minnows, obtained by dividing the MATC limits by the 96-h LC50 of 190 μg/ℓ (Table 5-b), is between 0.022 to 0.042; that for mysids is between 0.13 and 1.0. If the application factor for sheepshead minnows can be applied to more sensitive fishes, chronic effects may be expected at concentrations less than 1 μg/ℓ. Based on the mysid MATC, therefore, it is evident that a concentration of 0.44 μg EPN/ℓ in estuarine waters could cause acute effects to sensitive species, such as *P. duorarum* (96-h LC50 = 0.29 μg/ℓ; Table 5-b), and may cause chronic effects to *M. bahia* and to sensitive fishes.

Information obtained on the persistence of EPN in laboratory studies demonstrates the importance of fate data in attempting to predict the probability that EPN will remain in the marine environment for durations sufficient to produce acute effects, chronic effects, or result in significant bioaccumulation. Hydrolysis and biologically mediated degradation did occur in our tests. This information coupled with data on expected extent and duration of inputs from freshwater agricultural run-off may aid in predicting the likelihood of EPN reaching estuaries. If these predictions, or monitoring data, demonstrate that EPN would be present intermittently, then this insecticide's hazard relative to its chronic toxicity or bioaccumulation potential seems minimal. Chronic tests with *M. bahia* and *C. variegatus* demonstrated that effects occurred during the later stages of exposure; thus, chronic effects may be unlikely if EPN occurs intermittently. Although EPN is bioconcentrated, it is also rapidly depurated; thus, significant accumulation is unlikely unless EPN is present continuously. Our studies suggest that the acute toxicity of EPN to estuarine invertebrates is possibly the greatest hazard because death can occur at concentrations that cannot be detected.

ETHOPROP

Results

Acute Static Toxicity Tests

Algae--The 96-h EC50 for *Skeletonema costatum* exposed to ethoprop was 8,400 μg/ℓ (Table 4-b and Appendix C-1). In these tests many elongated cells were evident, possibly a result of inhibited cell division.

Invertebrates--The toxicity of ethoprop to embryos of eastern oysters (*Crassostrea virginica*) is shown in Table 4-b, Appendix H-1a, and H-1b. The 48-h EC50 was 16,000 μg/ℓ. The 96-h LC50 values and 95% confidence intervals for the crustaceans were 23 (19 to 27) μg/ℓ for *Mysidopsis bahia*, and 6.4 (5.5 to 7.4) μg/ℓ for *Penaeus stylirostris* (Table 4-b; Appendix H-2 and H-3).

Fishes--The 96-h LC50 and 95% confidence intervals of ethoprop for sheepshead minnows (*Cyprinodon variegatus*) was 740 (585 to 1,070) μg/ℓ; for spot (*Leiostomus xanthurus*) 33 (0 to 48) μg/ℓ. (Table 4-b, Appendix H-4, and Appendix H-5.)

Acute Flow-through Toxicity Tests

Invertebrates--Based on measured concentrations, the 96-h LC50 and 95% confidence intervals of ethoprop for mysid shrimp, was 7.5 (6.4 to 9.2) μg/ℓ (Table 5-b and Appendix H-6) and for pink shrimp (*Penaeus duorarum*) 13 (4.7 to 37) μg/ℓ (Table 5-b and Appendix H-7).

Fishes--The 96-h LC50 and 95% confidence intervals of ethoprop for the sheepshead minnow was 180 (85 to 390) μg/ℓ based on measured concentrations (Table 5-b and Appendix H-8) and for pinfish (*Lagodon rhomboides*) was 6.3 (4.8 to 8.4) μg/ℓ (Table 5-b and Appendix H-9).

Ranges of DO and pH measured in seawater used in most acute tests are in Appendix C-8.

Mysid Shrimp Entire Life-cycle Test

Mysid shrimp exposed to ethoprop in a 28-day test were adversely affected. From Day 21, to the end of the exposure, mortality was significantly greater in mysids exposed to the two highest concentrations (0.62 and 1.4 μg/ℓ; Table 42). Time to the formation of brood pouches appeared not to be affected by ethoprop at the concentrations tested (Table 42). The number of offspring produced per female was significantly reduced in the highest (1.4 μg/ℓ) concentration (Table 43).

There was no significant mortality of F_1 mysids during a post-hatch exposure of four to eight days. Survival ranged from 94 to 100% in each treatment.

The MATC of ethoprop, based on measured concentrations, was >0.36 <0.62 μg/ℓ and the application factor limits (MATC divided by the 96-h LC50 in a flow-through test) were 0.048-0.083.

Sheepshead Minnow Embryo/Juvenile Test

Sheepshead minnows exposed to ethoprop during their embryonic to juvenile stages were adversely affected. Exposure to 98 μg/ℓ produced no significant effect on time-to-hatch or hatching success compared with those in control aquaria, nor were abnormalities in embryonic development observed (Table 44). Exposure to 21 μg/ℓ significantly increased mortality of juvenile sheepshead minnow. By post-hatch, Day 7, approximately 83% of all fish that died during the entire 28-day test had died; and by Day 14, 95% of all deaths had occurred. No physical abnormalities were observed among juvenile fish in any treatment.

The growth of surviving juveniles was not significantly affected in concentrations ≤21 μg/ℓ (Table 45). Few fish survived concentrations ≥35 μg/ℓ, and they were not measured.

Ethoprop was detected in the tissues of sheepshead minnows that survived the 28-day exposure (Table 45). The tissue concentrations ranged from 0.1 to 0.48 μg/g; bioconcentration factors from 4 to 17.

The estimated MATC of ethoprop for embryos and juveniles of sheepshead minnows was >12<21 μg/ℓ. The 96-h LC50 for sheepshead minnows exposed to ethoprop was 180 μg/ℓ (Table 5-b); therefore, the application factor limits were 0.067-0.12.

Discussion

The results of toxicity tests with ethoprop indicate that estuarine crustaceans and fishes are sensitive at concentrations ≥6.3

≤740 µg/ℓ, and that oysters and a phytoplankter are probably less sensitive. Like phorate, the acute toxicity values of ethoprop to the majority of the fishes tested are similar to those of the crustaceans (Tables 4-b and 5-b). A comparison of the ethoprop static and flow-through tests on the same species indicates that the static results significantly underestimate ethoprop toxicity to mysid shrimp and sheepshead minnows. This may be due to a number of factors, including loss of the chemical in the exposure water by adsorption on glass, uptake by the test organism, and metabolism or alteration of the parent compound to a less toxic substance. Since no persistence studies have been conducted, no definitive explanation can be made.

Search of the literature produced very little additional data on the toxicity of ethoprop to aquatic organisms. The only information found was static acute toxicity data on the freshwater fish, rainbow trout (Salmo gairdneri), and the cladoceran (Daphnia magna) (U.S. EPA, 1975). The 96-h LC50 of concentrated (92%) ethoprop was 1,150 µg/ℓ and that of 10% granular material to Daphnia magna was 690,000 µg/ℓ. Reasons for the wide disparity in sensitivity to ethoprop between the freshwater and estuarine species are unclear. However, data collected from flow-through tests with freshwater species may permit explanation of these differences.

Chronic toxicity tests with the mysid shrimp and sheepshead minnow both demonstrated that the highest concentration that produced no significant deleterious effect in the long-term studies was not vastly different from concentrations that are lethal in acute tests (Tables 5-b, 42, and 43). It is interesting to note that although LC50s differed by a factor of 25, the range of the application factors overlapped (0.048 to 0.083 for mysids, 0.067 to 0.12 for sheepshead minnows). The time required to elicit lethal responses in each test differed. In the mysid test, significant differences in mortality were not observed until after 3 weeks exposure, whereas in the sheepshead minnow test, 83% of all total deaths occurred by post-hatch Day 7 and 95% by post-hatch Day 14. In both tests, however, mortality was the factor that determined the MATC.

METHYL PARATHION

Results

Acute Static Toxicity Tests

Algae--The 96-h EC50 and 95% confidence limits for Skeletonema costatum exposed to methyl parathion were 5,300 µg/ℓ, (4,300 to 5,700 µg/ℓ) (Table 4-b, and Appendix C-1). The no-effect concentration was 1,000 µg/ℓ.

Invertebrates--The calculated 48-h EC50 and 95% confidence limits for embryos of eastern oysters (Crassostrea virginica) exposed to methyl parathion was 12,000 (1,000 to 160,000 µg/ℓ) (Table 4-b, Appendix I-1a and I-1b). Reduction in numbers of embryos that developed normally to the straight-hinged, veliger stage after 48 h ranged from 25% in 3,200 μg/ℓ to 71% in 56,000 μg/ℓ.

Acute toxicity values of methyl parathion to three species of crustaceans are shown in Table 4-b, Appendix I-2, Appendix I-3, and Appendix I-4. The 96-h LC50's and 95% confidence intervals were 0.98 μg/ℓ (0.81 to 1.20) for mysid shrimp (*Mysidopsis bahia*), 1.4 μg/ℓ (1.3 to 1.6) for white shrimp (*Penaeus stylirostris*), and 28 μg/ℓ (16 to 49) for a copepod (*Acartia tonsa*). Mortality ranged from 0% in 0.32 μg/ℓ to 90% in 1.8 μg/ℓ for mysid shrimp, from 33% in 0.32 μg/ℓ (equal to control mortality) to 100% in 5.6 μg/ℓ for white shrimp, and from 20% in 13 μg/ℓ to 100% in 100 μg/ℓ for the copepod.

Fishes--The 96-h LC50s and 95% confidence intervals for two species of fishes were 12,000 μg/ℓ (10,000 to 14,000) for sheepshead minnows, *Cyprinodon variegatus*, and 93 μg/ℓ (56 to 320) for spot, *Leiostomus xanthurus*, (Table 4-b, Appendix I-5, and Appendix I-6). Mortality ranged from 0% in 10,000 μg/ℓ to 100% in 14,000 μg/ℓ for sheepshead minnows, and from 0% in 56 μg/ℓ to 100% in 320 μg/ℓ for spot.

Acute Flow-through Toxicity Tests

Invertebrates--The 96-h LC50's and 95% confidence intervals for two invertebrates tested were 0.77 μg/ℓ (0.65 to 0.98) for mysid shrimp and 1.2 μg/ℓ (0.91 to 1.4) for pink shrimp (*Penaeus duorarum*) (Table 5-b, Appendix I-7, and Appendix I-8). Methyl parathion was not detected in pink shrimp that survived the tests.

Fishes-- The 96-h LC50 for spot and 95% confidence limits were 59 μg/ℓ (45 to 74) (Table 5-b). Mortality ranged from 10% in 25 μg/ℓ to 100% in 250 μg/ℓ (Appendix I-9).

Ranges of DO and pH measured in seawater used in most acute tests are shown in Appendix C-8.

Mysid Shrimp Entire Life-cycle Test

Results of exposure of *M. bahia* to methyl parathion in a life-cycle test show that survival (Table 46) and reproductive success (number of progeny produced per female) (Table 47) are both reduced in 0.37 μg/ℓ. A low survival in the TEG carrier control group was attributed to excessive TEG (>50 mg/ℓ) being injected into this test chamber, which stimulated growth of algae and probably other micro-organisms that may have caused excessive death of mysids.

The MATC limits for methyl parathion are between 0.11 and 0.37 μg/ℓ. The application factor was 0.14 to 0.48.

Persistence

The Log P for methyl parathion is 2.4 (Table 26). If this value is applied to the Log P/Log BCF regression curve for fathead minnows (Veith, 1979a), the estimated BCF for methyl parathion is 22X. We conducted no long-term study to verify this result.

The results of persistence studies with methyl parathion are reported in Table 48. Only 3% remained in untreated sediment/water systems after 5 days. In contrast, EPN and methyl parathion remained in the sterilized sediment/water systems after 7-days. From these data, the implications are that biological processes are most important in the disappearance of methyl parathion. Examination of the fortified seawater systems indicate a less significant role for biological and nonbiological processes present in the water column, which substantiates that the biological component of the sediments is primarily responsible for the disappearance of methyl parathion in the untreated systems. Volatility of the pesticide was not a factor in the disappearance of methyl parathion in any of these systems since the resin tubes fails to reveal the presence of any pesticide. In the studies with natural sunlight (Sunlight), thermal factors did not affect disappearance since the foil-covered systems (Dark) did not differ from the seawater systems. Although natural sunlight, compared to artificial light, did enhance this pesticide's disappearance, biological processes in the sediments remain the major contributor.

Discussion

Methyl parathion was not very toxic to the alga, S. costatum (95-h EC50 = 5,300 μg/ℓ). We know of no other studies of the toxicity of methyl parathion to algae or other aquatic plants with which to compare our test (Table 4-b).

Invertebrates, particularly crustaceans, were more sensitive to methyl parathion than were fish. In our tests, the mysid shrimp were particularly susceptible. There was little difference in the results of acute and chronic exposures of mysids to methyl parathion or between the 96-h LC50s derived from static (0.98 μg/ℓ) and flow-through (0.77 μg/ℓ) tests. Two species of penaeid shrimp were almost as sensitive as mysid shrimp. The 96-h LC50 for P. stylirostris (static test) was 1.4 μg/ℓ; that for P. duorarum (flow-through test) 1.2 μg/ℓ. The copepod (A. tonsa) was the least sensitive (28 μg/ℓ static test) crustacean tested. Methyl parathion had relatively little effect on embryos of the oyster (C. virginica) (static test, 48-h EC50 = 12,000 μg/ℓ), representing the least sensitive to methyl parathion of all the species we tested.

Other studies on the toxicity of this compound to aquatic invertebrates appear to be limited to crustaceans. The range of acute toxicity of methyl parathion reported for other saltwater and freshwater crustaceans was from 2 to 40 μg/ℓ. In addition, concentrations lower than 1 μg/ℓ adversely affected the behavior of grass shrimp. Static 48-h LC50s for freshwater crayfish (*Procambarus clarki*) (Muncy and Oliver, 1963) were 40 μg/ℓ and for two populations of *P. acutus*, 2.4 and 3.4 μg/ℓ (Albaugh, 1972). Naqvi and Ferguson (1970), using 24-h static bioassays, found that the LC50s for four populations of the freshwater shrimp (*Palaemonetes kadiakensis*) ranged from 2.5 to 23.3 μg/ℓ. Saltwater crustaceans are equally sensitive to methyl parathion. In static tests, 96-h LC50s ranged from 2 to 7 μg/ℓ for sand shrimp (*Crangon septemspinosa*), grass shrimp (*Palaemonetes vulgaris*), and hermit crab (*Pagurus longicarpus*) (Eisler, 1969).

Farr (1977) determined that 0.1 μg methyl parathion/ℓ impairs the ability of grass shrimp (*Palaemonetes pugio*) to escape predation by the gulf killifish (*Fundulus grandis*). Even lower concentrations (0.024 μg/ℓ) altered the relative proportions of prey (grass shrimp and sheepshead minnows) in the diet of this predator (Farr, 1978).

Available data also indicate a wide range of toxicity of methyl parathion to fish. Our 96-h LC50s for spot (static, 93 μg/ℓ; flow-through, 59 μg/ℓ) are the lowest concentrations of this toxicant in water that have been reported to kill fish. Acute toxicity to the sheepshead minnow and to other saltwater and freshwater fish ranged from 1,900 μg/ℓ to 75,800 μg/ℓ. The 96-h LC50s for 19 species of freshwater fish in static tests ranged from 1,900 μg/ℓ for the bluegill (*Lepomis macrochirus*) to 15,200 μg/ℓ for the banded killifish (*Fundulus diaphanus*) (Pickering et al., 1962; Macek and McAllister, 1970; Rehwoldt et al., 1977). Sensitivity (96-h LC50 values) of 7 species of saltwater fish in static tests ranged from 5,200 μg/ℓ for the striped mullet (*Mugil cephalus*) to 75,800 μg/ℓ for the northern puffer (*Sphaeroides maculatus*) (Eisler, 1970).

The relationship of brain AChE inhibition to death of sheepshead minnows after exposures up to 72 h to methyl parathion was determined by Coppage (1972). The chemical reduced brain AChE activity to less than 17.7% of normal when 40 to 60% of the fish were killed.

Pathological changes also occur in fish following methyl parathion poisoning. McCann and Jasper (1972) observed hemorrhaging in bluegill exposed less than 6 h to concentrations that ranged from 3,900 to 6,900 μg/ℓ. Anees (1978) found that 1,050 μg/ℓ caused intestinal and liver lesions in the freshwater fish (*Channa punctatus*) in 96 h.

Data on the toxicity, bioaccumulation, and fate of methyl parathion must be compared if the hazard of this insecticide to the marine environment is to be assessed. Because crustaceans are particularly sensitive to acute and chronic effects of methyl parathion, they are more likely to be impacted than algae, other invertebrates, or fishes if this insecticide enters the marine environment. The results of our laboratory persistence tests and those using more complex laboratory systems (Pritchard et al., 1979) demonstrate that biological and chemical hydrolysis act to rapidly degrade methyl parathion. If this occurs at the same rate in estuaries and input is intermittent, it would seem unlikely that methyl parathion would persist for sufficient durtions for chronic exposures or bioaccumulation to occur.

PHORATE

Results

Acute Static Toxicity Tests

Algae--The 96-h EC50 and 95% confidence limits for *Skeletonema costatum* exposed to phorate were 1,300 µg/ℓ (1,000 to 1,400 µg/ℓ) (Table 4-b and Appendix C-1). Growth of this alga in 100 µg/ℓ did not differ from growth of control cultures.

Invertebrates--The 48-h EC50 of phorate to eastern oysters (*Crassostrea virginica*) was 900 µg/ℓ (400 to 1,900 µg/ℓ) (Table 4-b, Appendix J-1a, and Appendix J-1b).

In static tests, the 96-h LC50 was 0.27 µg/ℓ (0.18 to 0.32 µg/ℓ) for white shrimp postlarvae (*Penaeus stylirostris*) and 1.9 µg/ℓ (1.0 to 3.2 µg/ℓ) for mysid shrimp (*Mysidopsis bahia*) (Table 4-b, Appendix J-2, and Appendix J-3).

Fishes--The 96-h LC50s of phorate to two fishes were similar, 4.0 µg/ℓ for *C. variegatus* and 5.0 µg/ℓ for *L. xanthurus* (Table 4-b, Appendix J-4, and Appendix J-5). Survival decreased only slightly between Day 1 and Day 4 for each species during each test.

Acute Flow-through Toxicity Tests

Invertebrates--The 96-h LC50s of phorate, based on flow-through tests with measured concentrations in the exposure water, to pink shrimp (*Penaeus duorarum*) and mysid shrimp were less than 1 µg/ℓ (Table 5-b, Appendix J-6, and Appendix J-7); 0.33 (0.27 to 0.43) µg/ℓ for mysid shrimp, and 0.11 (0.08 to 0.16) µg/ℓ for pink shrimp. The LC50 for mysid shrimp in this flow-through test was only one-fifth of the static LC50 for that species and the 96-h LC50 for juvenile pink shrimp was two-fifths of the LC50 for postlarval white shrimp in a static test. These data suggest that chemical, physical, or biological factors are reducing the actual concentration of phorate in static tests, thus increasing the LC50.

Fishes--Sheepshead minnows were slightly more sensitive to phorate in flow-through acute tests than were spot (Table 5-b, Appendix J-8, and Appendix J-9). The 96-h LC50s based on chemically measured water concentrations were 1.3 (0.97 to 1.7) µg/ℓ for sheepshead minnows and 3.9 (3.1 to 5.6) µg/ℓ for spot. The LC50s for fishes were lower in flow-through than in static acute toxicity tests.

Ranges of DO and pH mesured in seawater used in most acute tests are shown in Appendix C-8.

Mysid Shrimp Entire Life-cycle Test

In an entire life-cycle toxicity test, mysid shrimp were exposed to phorate for 28 days. Survival, the most sensitive measure of effect, was significantly reduced in an average measured concentration of 0.21 µg/ℓ (Table 49), a concentration only slightly less than the 96-h LC50. Twenty-seven of the 30 mysids that died in this concentration did so in the first 8 days of exposure. There were no significant effects on reproduction at the concentrations tested (Table 50). Visual observations indicated that growth was probably not affected. We observed no statistically significant effects on survival or reproduction at an average measured concentration of 0.09 µg/ℓ. The MATC limits for phorate are between 0.09-0.21 µg/ℓ. The application factor is >0.29 <.68 of the 96-h LC50 from a flow-through test reported in Table 5-b. Therefore, the effect of phorate on mysid shrimp is acute, rather than chronic in nature.

Sheepshead Minnow Embryo-Juvenile Tests

There was no significant difference ($\alpha = 0.01$) between survival of control embryos and embryos exposed to phorate (Table 51). Survival of embryos to hatching ranged from 88.8% to 98.8% per treatment.

Phorate was toxic to juvenile sheepshead minnows at a measured concentration of 0.41 µg/ℓ, when the fish were continuously exposed for four weeks post-fertilization (Table 52 and Figure 5). All fish exposed to 2.4 µg/ℓ died within 96 h after hatching; 96% of the fish exposed to 1.2 µg/ℓ and 42.9% of those in 0.77 µg/ℓ died during the test. Mortality was significantly different between control fish and those exposed to 2.4, 1.2, and 0.77 µg/ℓ ($\alpha = 0.01$) and between control fish and those exposed to 0.41 µg/ℓ ($\alpha = 0.05$). Mortality of fish exposed to 0.41 µg/ℓ for 24 days was 2.5%, but by Day 28, had increased to 11.4% with some survivors visibly affected, indicating that further exposure could have increased mortality.

Time of occurrence and extent of signs of poisoning were dependent on duration of exposure. Initially, affected fish were lethargic with some developing lateral flexure of the body; thereafter, the body became thickened when viewed from above, fish failed to feed, lost equilibrium, and died. Within 24 h after hatching, all fish exposed to 2.4 µg/ℓ were lethargic and over 50% had visible flexure. Twenty-four hours later, over half the fish exposed to 2.4 µg/ℓ had died and those in 1.2 µg/ℓ were lethargic and some flexed. Deaths began in the 1.2 µg/ℓ exposure concentration 4 days after hatching and body thickening became apparent in survivors 3 days later. Lethargy and flexure were observed in fish exposed to 0.77 µg/ℓ four days after hatching; noticeable thickening occurred 3 days later, and deaths commenced 12 days after edema was first observed. The fish in 0.41 µg/ℓ were lethargic 5 days after hatching; flexure occurred intermittently, thickening was apparent 12 days after becoming lethargic, and some mortality occurred by the end of test. No signs of poisoning were observed in fish exposed to 0.16 or 0.24 µg/ℓ.

The three remaining juvenile fish in 1.2 µg/ℓ were significantly larger ($\alpha = 0.01$) than fish in all other aquaria (Table 52). This probably resulted from the greater availability of food and space per juvenile in the egg cups. When the number of fry-feeding days was used as a covariate, the adjusted lengths of these fish were not significantly different ($\alpha = 0.01$) from controls.

Phorate did not accumulate to any great extent in the tissues of sheepshead minnows. The BCF was 90 for juveniles that contained detectable phorate concentrations (Table 52).

Persistence

The Log P for phorate is 3.2 (Table 26). If this value is applied to the Log P/Log BCF regression curve for fathead minnows (Veith, 1979a), the estimated BCF of phorate is 100X. Although long-term BCF studies were not conducted to verify this result, juvenile sheepshead minnows after 28 days of exposure bioconcentrated phorate 90X.

The results of persistence studies with phorate (Table 53) demonstrate that phorate is less persistent than our reference pesticide, methyl parathion. We cannot determine the relative significance of biological and non-biological processes in the sediment/water systems because the pesticide was not detectable after five days in untreated systems or seven days in sterile systems. Volatility of phorate from sediment/water systems was not evident since analysis of resin tubes failed to reveal any pesticide. However, data from the seawater systems demonstrate that some disappearance of pesticide was the result of removal with exiting air. Since the purging rate is competitive with other processes affecting the disappearance of phorate in the seawater systems, the failure to account for the remaining pesticide in the water from the systems indicates that hydrolytic processes in the water column account for much of the phorate disappearance in these systems. The exact significance of sediments in the disappearance of phorate is not evident from these data, although the presence of sediment inhibited volatilization in both untreated and sterile systems.

Discussion

The results of acute toxicity tests with phorate indicate that fishes and crustaceans are particularly sensitive, but that oysters and phytoplankters may be less sensitive. Unlike the other three organophosphate insecticides, the acute toxicity of phorate to crustaceans and fishes seems similar. These conclusions are supported by our data (Tables 4-b and 5-b) and by the flow-through tests of Lowe (personal communication: 1980, Jack I. Lowe, U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida). Lowe found that the nominal concentration of phorate required to immobilize or kill 50% of the brown shrimp (Penaeus aztecus) in 48 h was 0.46 µg/ℓ and to reduce shell growth of the eastern oyster by 50% in 96 h was estimated to be 640 µg/ℓ. He also determined that the 48-h LC50 based on nominal concentrations for spot was 3.2 µg/ℓ; for longnose killifish (Fundulus similis) 0.36 µg/ℓ. Coppage (1972) exposed sheepshead minnows to 9 µg/ℓ for 48 h and observed 40 to 60% kill.

Chronic toxicity tests demonstrated that the highest concentration that caused no significant effect in entire life-cycle tests with mysid shrimp and in an embryo-juvenile test with sheepshead minnows differed only slightly from the 96-h LC50 for these species (Tables 5-b and 8). The only significant effect of phorate on mysids in the 28-day test was that survival was adversely affected during the first eight days. In contrast, sheepshead minnows died throughout the 28-day embryo/juvenile test, indicating that the lower limit on the MATC (0.24 to 0.41 µg/ℓ) may not be a "safe" concentration of phorate. However, embryo-juvenile tests for other fishes and chemicals have been shown to be good predictors of MATC's in entire life-cycle toxicity tests (McKim, 1977; Macek, 1977).

Examination of data on the toxicity, bioaccumulation, and persistence of phorate is necessary to determine the hazard of phorate to marine environments. Persistence tests with phorate indicate that this insecticide disappears from these laboratory systems more rapidly than EPN or methyl parathion. If this relationship is also true for estuaries, this could limit the expected environmental concentration, chronic effects, and bioaccumulation, especially if concentrations in freshwater run-off are low and intermittent. Even if concentrations in estuaries are low and occur intermittently, the hazard of phorate should not be minimized because intermittent exposure to concentrations less than 1 µg/ℓ would be expected to impact sensitive invertebrates and fish.

REFERENCES

Albauth, D.W. 1972. Insecticide tolerances of two crayfish populations (Procambarus acutus) in south-central Texas. Bull. Environ. Contam. Toxicol. 8:334-338.

American Society for Testing and Materials Committee E-35 on Pesticides. 1978. Standard practice for conducting static basic acute toxicity tests with larvae of four species of bivalve molluscs. Draft No. 4.

Anees, M.A. 1978. Hepatic pathology in a fresh-water teleost Channa punctatus (Bloch) exposed to sub-lethal and chronic levels of three organophosphorus insecticides. Bull. Environ. Contam. Toxicol. 19:524-527.

Bahner, L.H., and J.L. Oglesby. 1979. Test model for predicting Kepone accumulation in selected estuarine species. Aquatic Toxicology, ASTM STP 667. R.A. Kimerle and L.L. Marking, Editors, American Society for Testing and Materials. pp. 221-231.

Bahner, L.H., and J.L. Oglesby. Models for predicting Kepone^R accumulation and toxicity in laboratory exposures and natural ecosystems. In: Environment Risk Analysis for Chemicals. R.A. Conway, Editor. Van Nostrand Reinbold Co., N.Y. (In press.)

Bourquin, A.W., R.L. Garnas, P.H. Pritchard, F.G. Wilkes, C.R. Cripe, and N.I. Rubinstein. 1979. Interdependent microcosms for assessment of pollutants in the marine environment. Intl. J. Environ. Stud. 13:131-140.

Buchanan, D.V., M.J. Meyers, and R.S. Caldwell. 1975. Improved flowing water apparatus for the culture of brachyuran crab Fish. Res. Board Can. 32:1880-1883.

Butler, P.A. and R.L. Schultzman. 1978. Residues of pesticides and PCB's in estuarine fish, 1972-1976. Nat. Pestic. Monit. Prog., Pestic. Monit. J. 12(2):51-59.

Caldwell, R.S. 1977. Biological effects of pesticides on the Dungeness crab. EPA-600/3-77-131. U.S. Environmental Protection Agency (EPA), Gulf Breeze, FL. 125 p.

Caldwell, R.S., D.V. Buchanan, D.A. Armstrong, M.H. Mallon, and R.E. Millemann. 1979. Toxicity of the herbicides 2,4-D, DEF, Propanil and Trifluralin to the Dungeness crab, Cancer magister. Arch. Environ. Contam. Toxicol. 8:383-396.

Chiou, C.T., V.H. Freed, D.W. Schmedding, and R.L. Kohnert. 1977. Partition coefficient and bioaccumulation of selected organic chemicals. Environ. Sci. Technol. 11(5):475-478.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. EPA-600/3-75-009. U.S. Environmental Protection Agency, Washington, D.C. 61 p.

Coppage, D.L. 1971. Characterization of fish brain acetylcholinesterase with an automated pH-stat for inhibition studies. Bull. Environ. Contam. Toxicol. 6:304-310.

Coppage, D.L. 1972. Organophosphate pesticides: Specific levels of brain AChE inhibition related to death in sheepshead minnows. Trans. Am. Fish. Soc. 101:534-536.

Couch, J.A., J.T. Winstead, D.J. Hansen, and L.R. Goodman. 1979. Vertebral dysplasia in young fish exposed to the herbicide trifluralin. J. Fish Dis. 2:35-42.

Duangsawasdi, M. 1977. Organophosphate insecticide toxicity in rainbow trout (Salmo gairdneri). Effects of temperature and investigations on sites of action. Diss. Abstr. Int. B 38(11):5228.

Eisler, R. 1969. Acute toxicities of insecticides to marine decapod crustaceans. Crustaceana 16(3):302-310.

Eisler, R. 1970. Acute toxicities of organochlorine and organophosphorus insecticides to estuarine fishes. U.S. Fish Wildl. Serv. Bur. Sport Fish. Wildl. Tech. Pap. No. 46. 12 p.

Fabacher, D.L., J.D. Davis, and D.A. Davis. 1976. Apparent potentiation of the cotton defoliant DEF^R by methyl parathion in mosquitofish. Bull. Environ. Contam. Toxicol. 6:304-310.

Farr, J.A. 1977. Impairment of antipredator behavior in Palaemonetes pugio by exposure to sublethal doses of parathion. Trans. Am. Fish. Soc. 106:287-290.

Farr, J.A. 1978. The effect of methyl parathion on predator choice of two estuarine prey species. Trans. Am. Fish. Soc. 107:87-91.

Finney, D.J. 1971. Probit Analysis. Cambridge University Press; London. 333 p.

Goodman, L.R., D.J. Hansen, D.L. Coppage, J.M. Moore, and E. Matthews. 1979. Diazinon^R: Chronic toxicity to, and brain acetylcholinesterase inhibition in, the sheepshead minnow, Cyprinodon variegatus. Trans. Am. Fish. Soc. 108:479-488.

Hansen, D.J., S.C. Schimmel, and J. Forester. 1977. Endrin: Effects on the entire life-cycle of a saltwater fish, *Cyprinodon variegatus*. J. Toxicol. Environ. Health. 3:721-733.

Jensen, J.P. 1958. The relation between body size and number of eggs in marine malacostrea. Medd. Dan. Fish. Havunders. 2(19): 1-28.

Karickhoff, S.W., D.S. Brown, and T.A. Scott. 1979. Sorption of hydrophobic pollutants on natural sediments. Water Res. 13: 241-248.

Klaverkamp, J.F., B.R. Holden, and S.E. Harrison. 1975. Acute lethality and *in vitro* brain cholinesterase inhibition of acephate and fenitrothion in rainbow trout. Proc. West. Pharmacol. Soc. 18:358-361.

Korn, S., and R. Earnest. 1974. Acute toxicity of twenty insecticides to striped bass, *Morone saxatilis*. Calif. Fish Game 60:128-131.

Lyons, D.B., C.H. Buckner, B.B. McLeod, and K.M.S. Sundaram. 1976. The effects of fenitrothion, Metacil^{R}, and Orthene^{R} on frog larvae. Report CC-X-129. Chemical Control Research Institute, Ottawa, Canada. 86 p.

Macek, K.J., and W.A. McAllister. 1970. Insecticide susceptibility of some common fish family representatives. Trans. Am. Fish. Soc. 99:20-27.

Macek, K.J., and B.H. Sleight, III. 1977. Utility of toxicity tests with embryos and fry of fish in evaluating hazards associated with chronic toxicity of chemicals to fishes. In: Aquatic Toxicology and Hazard Evaluation. Proceedings of the First Annual Symposium on Aquatic Toxicology. F.L. Mayer and J.L. Hamelink, Editors, American Society for Testing and Materials. pp. 137-146.

McCann, J.A., and R.L. Jasper. 1972. Vertebral damage to bluegills exposed to acutely toxic levels of pesticides. Trans. Am. Fish. Soc. 101:317-322.

McKim, J.M. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Board Can. 34:1184-1154.

Midwest Research Institute. 1975. Initial Scientific and Minieconomic Review of Aldicarb. EPA-540/1-75-013, U.S. Environmental Protection Agency, Washington, D.C. 122 p.

Miyasaka, H., and N. Kabayashi. 1974. Three newly registered agricultural chemicals. Shokubutsu Boeki (Plant Protec.) 28(4):168-169.

Mount, D.I., and W. Brungs. 1967. A simplified dosing apparatus for fish toxicity studies. Water Res. 2:21-29.

Muncy, R.J., and A.D. Oliver, Jr. 1963. Toxicity of ten insecticides to the red crawfish, *Procambarus clarki* (Girard). Trans. Am. Fish Soc. 92:428-431.

Naqvi, S.M., and D.E. Ferguson. 1970. Levels of insecticide resistance in freshwater shrimp, *Palaemonetes kadiakensis*. Trans. Am. Fish. Soc. 99:696-699.

Neter, J., and W. Wasserman. 1974. *Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Designs*. Richard D. Irwin, Inc., Homewood, IL. 1-842.

Nimmo, D.R., L.H. Bahner, R.A. Rigby, J.M. Sheppard, and A.J. Wilson, Jr. 1977. *Mysidopsis bahia*: An estuarine species suitable for life-cycle toxicity tests to determine effects of a pollutant. Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, F.L. Mayer and J.L. Hamelink, Editors, American Society for Testing and Materials. pp. 109-16.

Parrish, P.R., E.E. Dyar, M.A. Lindberg, C.M. Shanika, and J.M. Enos. 1977. Chronic toxicity of methoxychlor, malathion, and carbofuran to sheepshead minnows (*Cyprinodon variegatus*). United States Environmental Protection Agency, Ecol. Res. Series EPA-600/3-77-059. 36 pp.

Parrish, P.R., E.E. Dyar, J.M. Enos, and W.G. Wilson. 1978. Chronic toxicity of chlordane, trifluralin, and pentachlorophenol to sheepshead minnows (*Cyprinodon variegatus*). United States Environmental Protection Agency, Ecol. Res. Series EPA-600/3-78-010. 53 pp.

Pickering, Q.H., C. Henderson, and A.E. Lemke. 1962. The toxicity of organic phosphorus insecticides to different species of warm-water fishes. *Trans. Am. Fish. Soc.* 91, 175-84.

Pritchard, P.H., A.W. Bourquin, H.L. Frederickson, and T. Maziarz. 1979. System Design Factors Affecting Environmental Fate Studies in Microcosms. In: "Microbial Degradation of Pollutants in Marine Environments," P.H. Pritchard and A.W. Bourquin, Editors., EPA-600/9-79-012. pp. 251-272.

Rabeni, C.F. and J.G. Stanley. 1979. Operational spraying of acephate to supress spruce budworm has minor effects on stream fishes and invertebrates. Bull. Environ. Contam. Toxicol. 23: 327-334.

Rehwoldt, R.E., E. Kelley and M. Mahoney. 1977. Investigations into the acute toxicity and some chronic effects of selected herbicides and pesticides on several freshwater fish species. Bull. Environ. Contam. Toxicol. 18:361-365.

Sanders, H.O. 1969. Toxicity of pesticides to the crustacean *Gammarus lacustris*. U.S. Bur. Sport Fish. Wildl. Tech. Pap. No. 25.

Sanders, H.O. 1972. Toxicity of some insecticides to four species of malacostracan crustaceans. U.S. Bur. Sport Fish. Wildl. Tech. Pap. No. 66. 19 p.

Schimmel, S.C., P.R. Parrish, D.J. Hansen, J.M. Patrick, Jr., and J. Forester. 1974. Endrin: Effets on several estuarine organisms. Proc. 28 Annu. Conf. Southeast. Assoc. Game Fish Comm. 187-194.

Schimmel, S.C., J.M. Patrick, Jr., and A.J. Wilson, Jr. 1977. Acute toxicity to and bioconcentration of endosulfan by estuarine animals. In: Aquatic Toxicology and Hazard-Evaluation. Proceedings of the First Annual Symposium Aquatic Toxicology. F.L. Mayer and J.L. Hamelink, Editors, American Society for Testing and Materials. p. 241-252.

Steel, R.G.D., and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York. 481 p.

Strickland, J.D.H., and T.R. Parsons. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Board Canada. 167:21-26.

Tagatz, M.E., J.M. Ivey, H.K. Lehman, and J.L. Oglesby. 1979. Effects of Sevin on development of experimental estuarine communities. Journ. Toxicol. Environ. Health 5:643-651.

Tyler-Schroeder, D.B. 1978a. Culture of the grass shrimp (*Palaemonetes pugio*) in the laboratory. In: Bioassay Procedures for the Ocean Disposal Permit Program. EPA-600/9-78-010, U.S. Environmental Protection Agency, Gulf Breeze, FL. pp. 69-71.

Tyler-Schroeder, Dana Beth. 1978b. Entire life-cycle toxicity test using grass shrimp (*Palaemonetes pugio* Holthuis). In: Bioassay Procedures for the Ocean Disposal Permit Program. EPA-600/9-78-010. pp. 83-88.

Tyler-Schroeder, D.B. 1979. Use of the grass shrimp (*Palamonetes pugio*) in a life-cycle toxicity test. In: *Aquatic Toxicology*, ASTM STP 667, L.L. Marking and R.A. Kimerle, Editors, American Society for Testing and Materials, Philadelphia, PA. pp. 159-170.

U.S. Department of the Interior. 1979. Semi-Annual Report of Research Highlights. U.S. Fish and Wildlife Service. Columbia National Fisheries Research Laboratory, Columbia, MO. 21 pp.

U.S. Environmental Protection Agency. 1975. Report on toxicity of ethoprop to rainbow trout (*Salmo gairdneri*) and *Daphnia magna*. Chemical and Biological Investigations Branch, Beltsville, Maryland.

Veith, G.D., D.L. Defor, and B.V. Bergstedt. 1979a. Measuring and estimating the bioconcentration factors of chemicals in fish. J. Fish. Res. Board Can. 36(9):1040-1048.

Veith, G.D., Austin, N.M., and Morris, R.T. 1979b. A rapid method for estimating log P for organic chemicals. Water Res. 13:43-47.

Walsh, G.E., and S.V. Alexander. 1980. A Marine Algal Bioassay Method: Results with Pesticides and Industrial Wastes. Water Air Soil Pollut. 13:45-55.

Williams, D.A. 1971. A test for the differences between treatment means when several dose levels are compared with a zero dose control. Biometrics 27:103-117.

Woelke, C.E. 1972. Development of a receiving water quality bioassay criterion based on the 48-h Pacific oyster (*Crassostrea gigas*) embryo. U.S. Bur. Commer. Fish. Tech. Report No. 9. 93 pp.

Yonezawa, Y. and Urushigawa, Y. 1979. Chemical-biological interactions in biological purification systems. V. Relation between biodegradation rate constants of aliphatic alcohols by activated sludge and their partition coefficients in a 1-octanol-water-system. Chemosphere 3, 139-142.

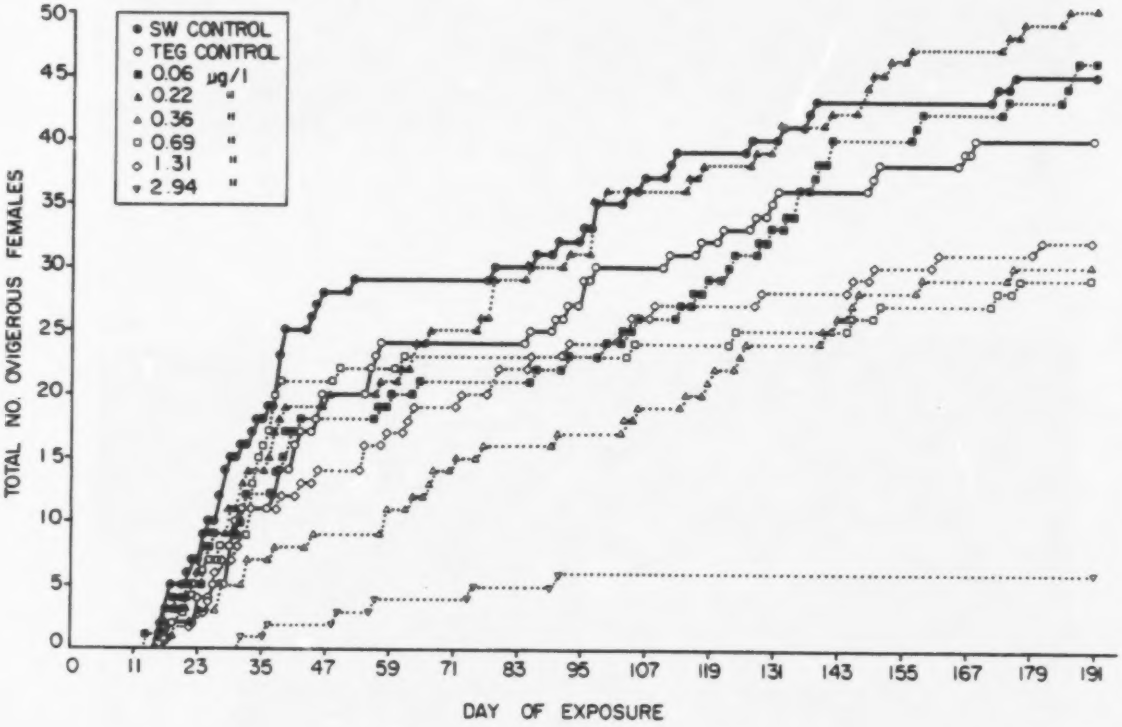


Figure 1. Effects of carbophenothion on spawning response of grass shrimp (Palaemonetes pugio) in a partial life-cycle toxicity test. The number of new ovigerous females in each exposure chamber was recorded daily during the test. Data were analyzed for differences in onset and duration of spawning and total number of females to spawn per concentration.

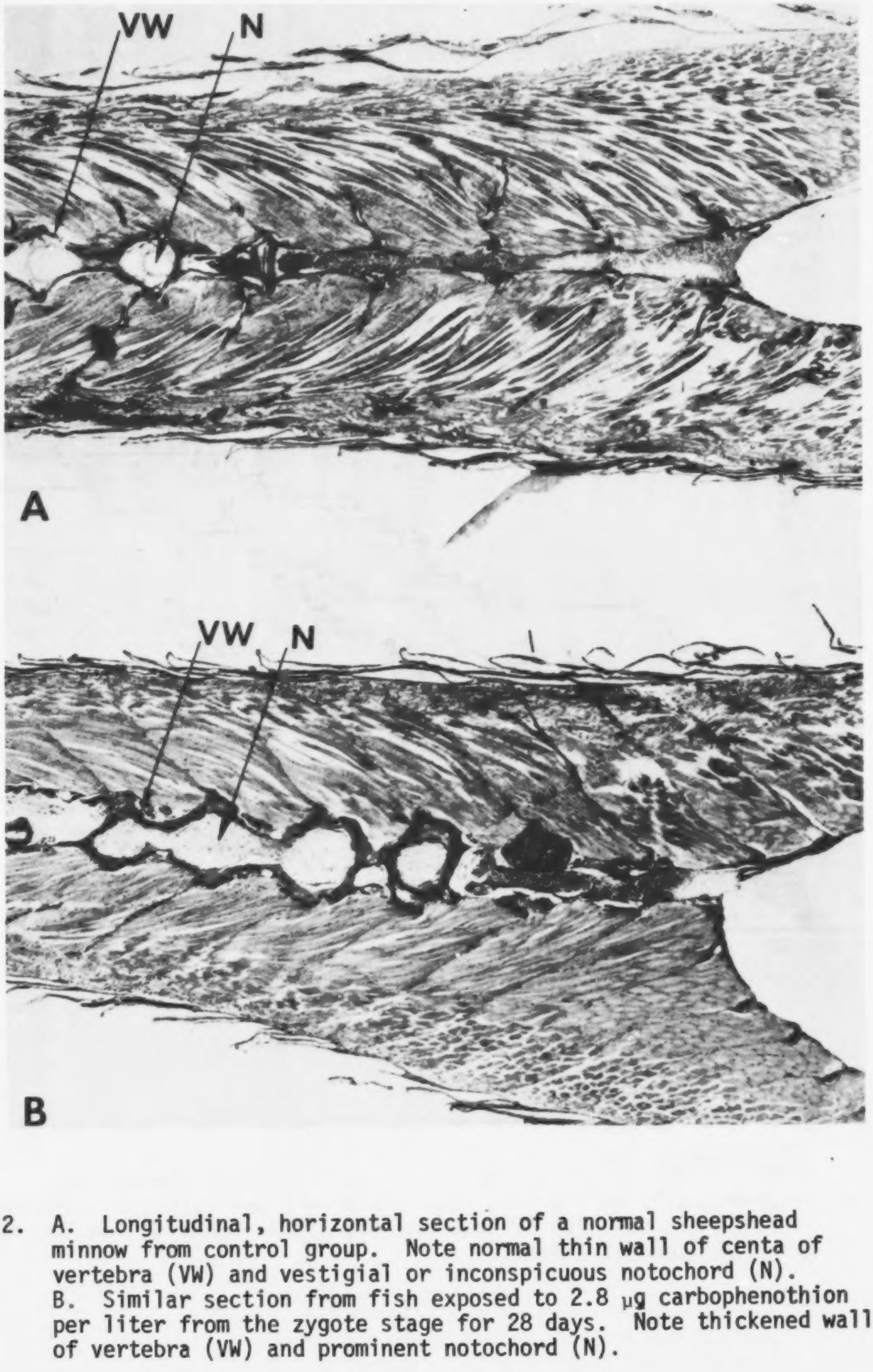


Figure 2. A. Longitudinal, horizontal section of a normal sheepshead minnow from control group. Note normal thin wall of centa of vertebra (VW) and vestigial or inconspicuous notochord (N). B. Similar section from fish exposed to 2.8 μg carbophenothion per liter from the zygote stage for 28 days. Note thickened wall of vertebra (VW) and prominent notochord (N).

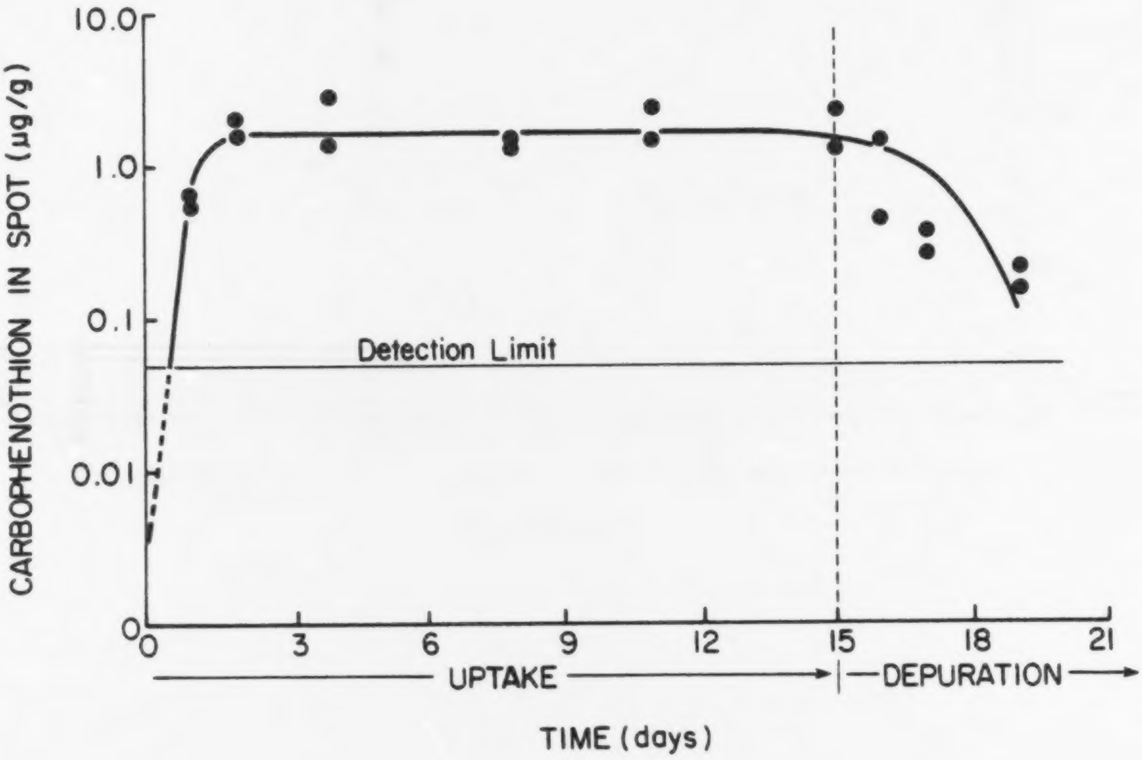


Figure 3. Uptake and depuration of carbophenothion in whole-body tissues of spot (*Leiostomus xanthurus*) in a flowing seawater bioassay. Exposure concentration averaged 2.9 µg carbophenothion per liter seawater.

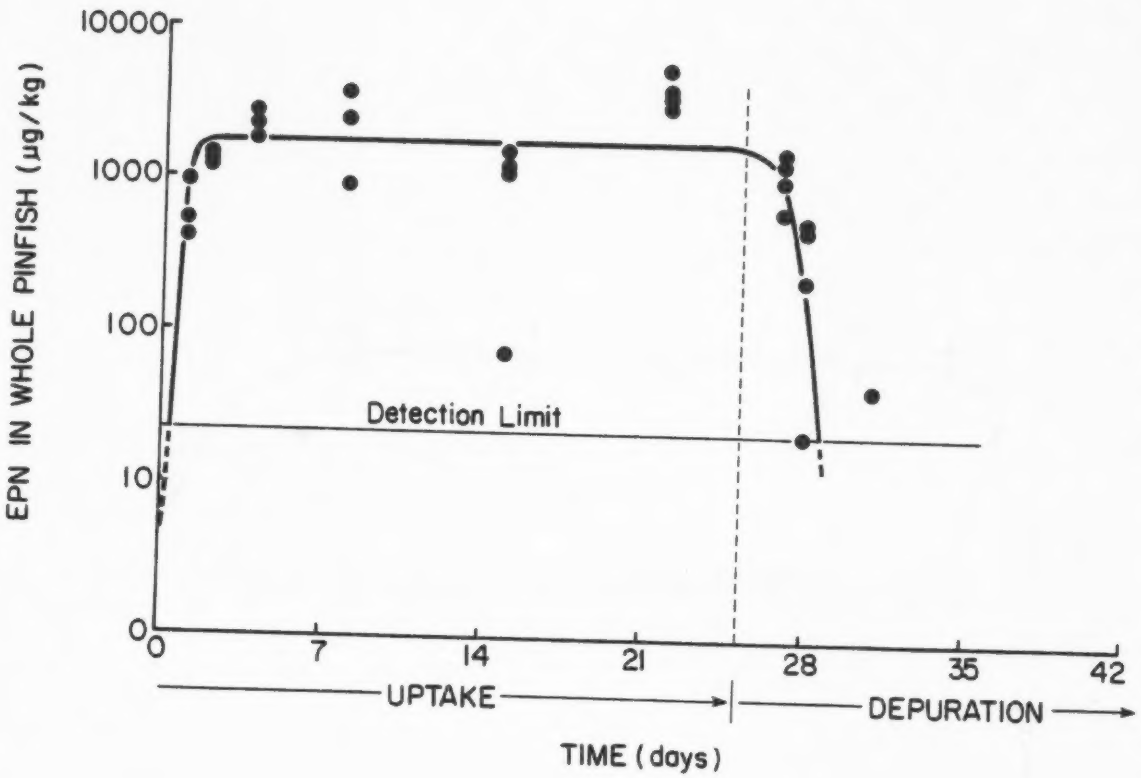


Figure 4. Uptake and depuration of EPN in whole-body tissues of pinfish (Lagodon rhomboides) in a flowing seawater bioassay. Exposure concentration averaged 2.4 µg EPN per liter seawater.

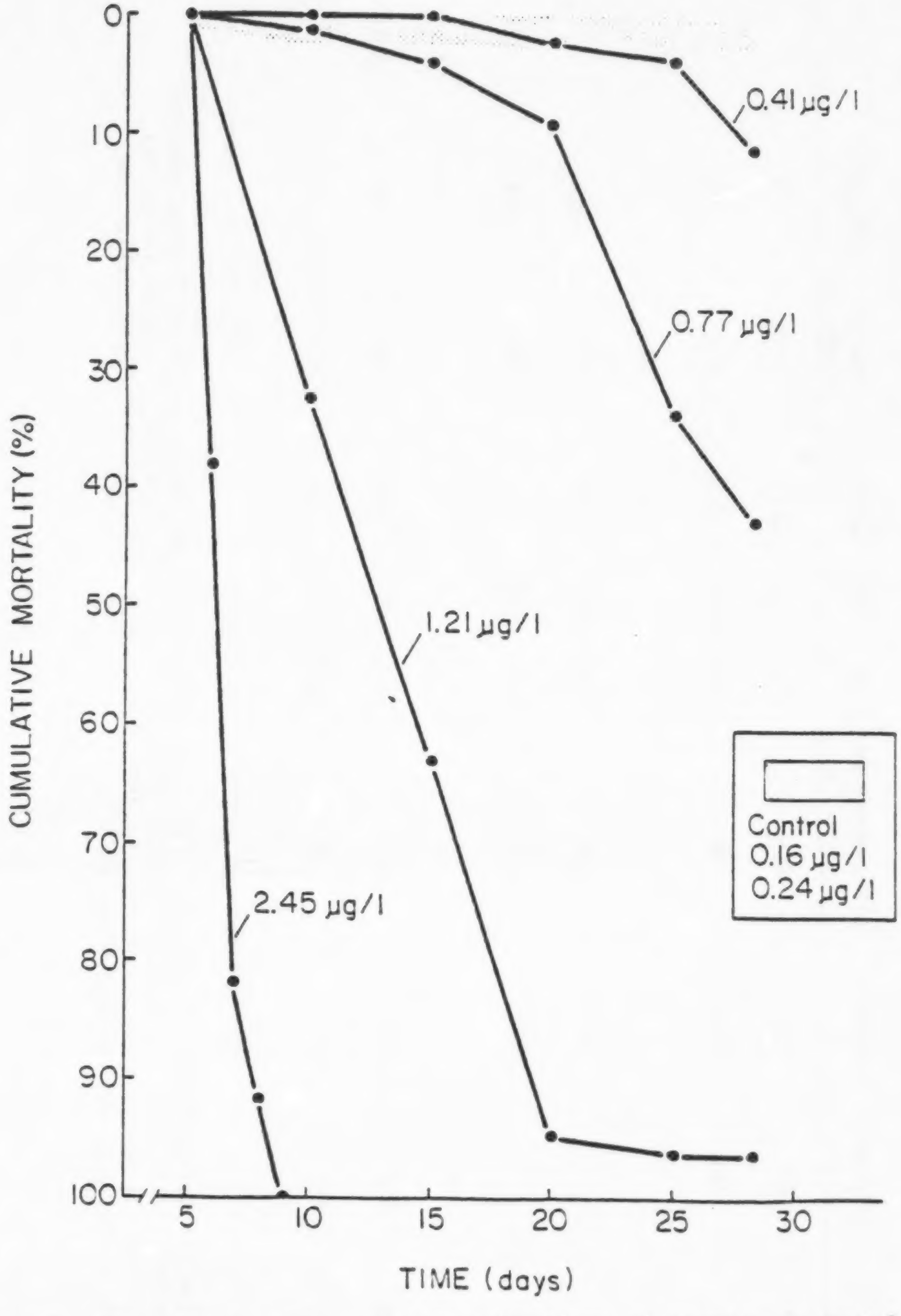


Figure 5. The effect of measured concentrations of phorate on mortality of sheepshead minnows (Cyprinodon variegatus) in a 28-day embryo/juvenile toxicity test at 30°C. Mortality for control fish and for fish in 0.16 and 0.24 µg per liter is within the shaded area.

Table 1-a. Scientist or laboratory responsible for conducting acute static toxicity tests.*

Species	Acephate	Aldicarb	Carbophenothion	DEF	EPN	Ethoprop	Methyl parathion	Phorate
Skeletonema costatum marine diatom	GEW	GEW	GEW	GEW	GEW	GEW	GEW	GEW
Crassostrea virginica eastern oyster	BMRL	BMRL	PWB	BMRL	BMRL	BMRL	BMRL	BMRL
Acartia tonsa marine copepod	-	-	-	-	-	-	BMRL	-
Mysidopsis bahia mysid shrimp	-	PWB	PWB	PWB	PWB	PWB	PWB	PWB
Penaeus stylirostris white shrimp	-	PWB	PWB	PWB	PWB	PWB	PWB	PWB
Penaeus duorarum pink shrimp	PWB	-	-	-	-	-	-	-
Cyprinodon variegatus sheepshead minnow	PWB	PWB	PWB	PWB	PWB	PWB	PWB	PWB
Leiostomus xanthurus spot	PWB	PWB	PWB	PWB	PWB	PWB	PWB	PWB

*GEW is an abbreviation for Gerald E. Walsh, U.S. EPA, ERL, Gulf Breeze, Florida; BMRL, Bionomics Marine Research Laboratory, EG&G International, Inc., Pensacola, Florida (Patrick R. Parrish, Director; P. Thomas Heitmuller, Terry A. Hollister, Frank I. Saksa, and G. Scott Ward); PWB, Patrick W. Borthwick (Kimberly J. Butler and Richard A. Zern), U.S. EPA, ERL, Gulf Breeze, Florida.

Table 1-b. Scientist or laboratory responsible for conducting acute flow-through toxicity tests.*

Species	Acephate	Aldicarb	Carbophenothion	DEF	EPN	Ethoprop	Methyl parathion	Phorate
Mysidopsis bahia	BMRL	BMRL	BMRL	DWN	DWN	BMRL	DWN	SCS
Penaeus duorarum	BMRL	BMRL	SCS	SCS	SCS	BMRL	SCS	SCS
Palaemonetes pugio grass shrimp	-	-	SCS	SCS	-	-	-	-
Cyprinodon variegatus	BMRL	BMRL	BMRL	SCS	SCS	BMRL	-	SCS
Menidia menidia Atlantic silverside	-	-	SCS	-	-	-	-	-
Lagodon rhomboides pinfish	BMRL	BMRL	BMRL	SCS	SCS	BMRL	-	-
Leiostomus xanthurus	-	-	SCS	SCS	SCS	-	BMRL	SCS

*BMRL is defined in Table 1-a. SCS is an abbreviation for Steven C. Schimmel (James M. Patrick, Jr.), U.S. EPA, ERL, Gulf Breeze, Florida; DWN, Del Wayne Nimmo (Timothy L. Hamaker, Charnell A. Sommers, and Roger Wood), U.S. EPA, ERL, Gulf Breeze, Florida.

Table 1c. Scientist or laboratory responsible for designated tests and sections of this document.*

Species	Acephate	Aldicarb	Carbophenothion	DEF	EPN	Ethoprop	Methyl parathion	Phorate
Embryo/juvenile								
Cyprinodon variegatus	-	BMRL	DJH	-	-	BMRL	-	DJH
Entire life-cycle								
Mysidopsis bahia	BMRL	BMRL	DBT	DWN	DWN	BMRL	DWN	DWN
Palaemonetes pugio	-	-	DBT	-	-	-	-	-
Cyprinodon variegatus	-	BMRL	-	-	DJH	-	-	-
Benthic community	-	-	MET	-	-	-	-	-
Bioconcentration								
Lagodon rhomboides	-	-	-	-	SCS	-	-	-
Leiostomus xanthurus	-	-	SCS	-	-	-	-	-
Persistence	-	-	RLG	RLG	RLG	-	RLG	RLG
Contributor	PWB	DBT	DJH	PWB	SCS	SCS	MET	DJH

*BMRL and PWB are defined in Table 1a. SCS and DWN are defined in Table 1b. DJH is an abbreviation for David J. Hansen (Larry R. Goodman, Peggy K. Higdon, Charles S. Manning, and Edward Matthews); DBT, Dana Beth Tyler (J.V. Wheat and R.D. Fink); MET, Marlin E. Tagatz (Joel M. Ivey); RLG, Richard L. Garnas (Jerrold Forester, Johnnie Knight, and James C. Moore); U.S. EPA, ERL, Gulf Breeze, Florida.

Table 2. Composition of mixes added to algal growth media.

Nutrient Mixes	Amount/Liter
Metal Mix[a]	
$FeCl_3.6H_2O$	0.048 g
$MnCl_2.4H_2O$	0.144 g
$ZnSO_4.7H_2O$	0.045 g
$CuSO_4.5H_2O$	0.157 mg
$CoCl_2.6H_2O$	0.404 mg
H_3BO_3	0.140 g
Na_2EDTA[b]	1.0 g
Glass-distilled or deionized water	1 ℓ
Vitamin Mix[c]	
Thiamin hydrochloride	50 mg
Biotin	0.01 mg
B_{12}	0.01 mg
Glass-distilled or deionized water	100 mℓ
Minor Salt Mix[d]	
K_3PO_4	3.0 g
$NaNO_3$	50.0 g
$Na_2SiO_3.9H_2O$	20.0 g
Glass-distilled or deionized water	1 ℓ

[a] Add 15.0 mℓ/ℓ of test solution.
[b] Added to stock culture medium for all pesticides except aldicarb.
[c] Add 0.5 mℓ/ℓ of test solution.
[d] Add 1.0 mℓ/ℓ of test solution.

Table 3. Profile of sediment used in persistence studies.

Sediment profile[a]	Percentage
Particle size[b] (mm)^2	
2.0 - 0.05	18.1
0.05 - 0.005	9.5
0.005 - 0.002	15.3
<0.002	57.1
Organic matter[c]	40.0
Moisture content[d]	89.6

[a] Range Point saltmarsh on Santa Rosa Island, near Pensacola Beach, Florida.

[b] Settling Rate-Soil Hydrometer Method (ASTM D422-63, 1964).

[c] Total organic matter on ignition at 500°C for 4 h.

[d] Twenty-five g sample of sediment dried on a steam table to constant weight.

Table 4-a. Acute toxicity of acephate, aldicarb, carbophenothion, and DEF to estuarine organisms in static tests. Values are 96-h LC50s, except where indicated. Nominal concentrations are in µg/ℓ and 95% confidence intervals are in parentheses.

Species	Acephate	Aldicarb	Carbophenothion	DEF
Skeletonema costatum[a] diatom	>50,000	>50,000	109 (100-115)	366 (315-391)
Crassostrea virginica[b] eastern oyster larvae	150,000 (8,000-300,000)	8,800 (1,400-56,000)	99 (96-102)	197 (120-323)
Mysidopsis bahia mysid shrimp 24-h-old	-	13 (10-15)	13 (10-17)	5.8 (4.4-6.8)
Penaeus stylirostris[c] white shrimp post larvae	-	72 (65-82)	4.3 (3.8-5.1)	25 (16-49)
Penaeus duorarum pink shrimp field collected	>10,000	-	-	-
Cyprinodon variegatus sheepshead minnows 28-day-old	>3,200,000	168 (102-320)	17 (14-21)	440 (410-473)
Leiostomus xanthurus spot	>100,000	202 (116-293)	500 (390-630)	158 (135-320)

[a] 96-h EC50 values.

[b] 48-h EC50 values.

[c] *P. stylirostris* post-larval stages were used in these tests; control mortality exceeded 10%.

Table 4-b. Acute toxicity of EPN, ethoprop, methyl parathion, and phorate to estuarine organisms in static tests. Values are 96-h LC50s, except where indicated. Nominal concentrations are in µg/ℓ and 95% confidence intervals are in parentheses.

Species	EPN	Ethoprop	Methyl parathion	Phorate
Skeletonema costatum[a] diatom	340 (330-350)	8,400 (7,600-9,000)	5,300 (4,300-5,700)	1,300 (1,100-1,400)
Crassostrea virginica[b] eastern oyster larvae	2,200 (600-8,100)	16,000 (7,000-38,000)	12,000 (1,000-160,000)	900 (400-1,900)
Acartia tonsa copepod	-	-	28 (16-49)	-
Mysidopsis bahia mysid shrimp 24-h-old	13 (10-18)	23 (19-27)	0.98 (0.81-1.2)	1.9 (1.0-3.2)
Penaeus stylirostris[c] white shrimp post larvae	4.6 (2.4-9.0)	6.4 (5.5-7.4)	1.4 (1.3-1.6)	0.27 (0.18-0.32)
Cyprinodon variegatus sheepshead minnows 28-day-old	140 (59-500)	740 (585-1,070)	12,000 (10,000-14,000)	4.0 (3.5-4.5)
Leiostomus xanthurus spot	37 (33-40)	33 (0-48)	93 (56-320)	5.0 (4.2-5.6)

[a] 96-h EC50 values.

[b] 48-h EC50 values.

[c] *P. stylirostris* post-larval stages were used in these tests; control mortality exceeded 10%.

Table 5-a. Acute toxicity of acephate, aldicarb, carbophenothion, and DEF to estuarine organisms in flowing seawater tests. Values are 96-h LC50s, except where indicated. Measured concentrations are in µg/ℓ and 95% confidence intervals are in parentheses.

Species	Acephate	Aldicarb	Carbophenothion	DEF
Mysidopsis bahia mysid shrimp	7,300 (300-190,000)	16 (13-20)	3.0 (2.4-3.7)	4.6 (4.2-4.9)
Penaeus duorarum pink shrimp	3,800 (2,000-7,300)	12 (7.5-18)	0.47 (0.36-0.66)	14 (10-18)
Palaemonetes pugio grass shrimp	-	-	4.6 (3.8-6.2)	22 (19-23)
wildstock larvae, 1-7 day	-	-	2.0 (1.8-2.6)	-
lab-reared, 49-day	-	-	2.9 (2.5-3.3)	-
Cyprinodon variegatus sheepshead minnow	910,000[a]	41 (55-72)	2.8 (0.85-9.6)	>440
Menidia menidia Atlantic silversides	-	-	7.7 (5.7-12.)	- -
Lagodon rhomboides pinfish	85,000 (7,800-933,000)	80 (43-150)	7.7 (4.6-12)	290 (240-379)
Leiostomus xanthurus spot	-	-	>206	130 (100-170)

[a] 96-h EC50 (nominal concentrations) based on loss of equilibrium.

Table 5-b. Acute toxicity of EPN, ethoprop, methyl parathion, and phorate to estuarine organisms in flowing seawater tests. Values are 96-h LC50s; measured concentrations are in µg/ℓ and 95% confidence intervals are in parentheses.

Species	EPN	Ethoprop	Methyl parathion	Phorate
Mysidopsis bahia mysid shrimp	3.4 (2.5-5.8)	7.5 (6.4-9.2)	0.77 (0.65-0.98)	0.33 (0.27-0.43)
Penaeus duorarum pink shrimp	0.29 (0.1-1.1)	13 (4.7-37)	1.2 (0.91-1.4)	0.11 (0.08-0.16)
Cyprinodon variegatus sheepshead minnow	190 (150-260)	180 (85-390)	-	1.3 (0.97-1.7)
Lagodon rhomboides pinfish	18 (15-24)	6.3 (4.78-8.4)	-	-
Leiostomus xanthurus spot	26 (19-24)	-	59 (45-74)	3.9 (3.1-5.6)

Table 6. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to acephate. The mysids were 24- to 48-h-old at the beginning of the test. Twenty mysids were placed in each treatment (10 per replicate). Salinity was from 26-31 °/oo and temperature, 22 ± 1°C.

Day	Measured concentration (µg/ℓ)											
	Control		580		520		1,400		2,000		4,200	
	A	B	A	B	A	B	A	B	A	B	A	B
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7[a]	0	0	0	0	0	0	0	10	0	0	10[b]	20[b]
8	0	0	0	0	0	0	10	10	0	0	10	20
9	0	0	0	0	0	0	10	10	0	0	10	20
10	0	0	0	0	0	0	10	10	0	0	10	20
11	0	0	0	0	0	0	10	10	0	0	10	20
12	0	0	0	0	0	0	10	10	0	0	10	20
13	0	0	0	0	0	0	10	10	0	0	10	20
14[a]	0	0	0	0	0	0	10[b]	10[b]	10[b]	10[b]	10[b]	30[b]
15	0	0	0	0	0	0	10	10	10	20	10	30
16	0	0	0	0	0	0	10	20	10	20	10	30
17	0[c]	0[c]	0[c]	0	0	0	10	20	10	20	10	30
18	0	0	0	0[c]	0[c]	0[c]	10[c]	20[c]	10[c]	20[c]	20	40
19	0	0	0	0	0	0	10	20	10	20	20	40[c]
20	0	0	0	0	0	0	10	20	10	20	20[c]	40
21[a]	0	0	0	0	0	0	10[b]	30[b]	10[b]	20[b]	20[b]	50[b]
22	0	0	0	0	0	0	10	30	10	20	20	60
23	0	0	0	0	0	0	10	30	10	20	30	60
24	0	0	0	0	0	0	10	40	10	20	30	60
25	0	0	0	0	10	0	10	40	10	20	40	60
26	0	0	0	0	10	0	10	40	10	20	40	60
27	0	0	0	0	20	0	10	40	10	20	40	60
28[a]	0	0	0	0	20	0	10[b]	40[b]	10[b]	20[b]	40[b]	60[b]
Average	0		0		10		25[b]		15[b]		50[b]	

[a] Statistical analysis performed.
[b] Significantly greater (α = 0.05) than the control.
[c] Formation of brood pouches first observed.

Table 7. Number of offspring produced per female of mysid shrimp (*Mysidopsis bahia*) exposed to acephate in a chronic (28-day) exposure in natural, flowing seawater.

Measured concentration (µg/ℓ)	Replicates		Total offspring	Females with brood pouches	Offspring per female
	A	B			
Control, ND[a]	8.8	8.0	76	9	8.4
580	8.8	5.3	60	8	7.5
520	7.3	6.0	46	7	6.6
1,400	7.0	5.3	51	8	6.4
2,000	4.8	5.0	49	10	4.9[b]
4,200	3.5	3.3	17	5	3.4[b]

[a] ND = nondetectable (<400 µg/ℓ).

[b] Significantly less ($\alpha = 0.05$) than the control as determined by ANOVA and Williams' test (Williams, 1971).

Table 8. Results of chronic toxicity tests with acephate, aldicarb, carbophenothion, DEF, EPN, ethoprop, methyl parathion, and phorate. The maximum acceptable toxicant concentration (MATC) range is the highest concentration (µg/ℓ) that produced no significant effect and the lowest concentration in which significant effects were observed. The application factor (AF) is the MATC range divided by the 96-h LC50 value from a flowing toxicity test.

Pesticide	*Mysidopsis bahia*[a]		*Palaemonetes pugio*[b]		*Cyprinodon variegatus*[c]	
	MATC	AF	MATC	AF	MATC	AF
Acephate	580-1,400	0.079-0.19	-	-	-	-
Aldicarb	1.0-1.5	0.063-0.094	-	-	50-88	-
Carbophenothion	0.48-1.2	0.16-0.40	0.22-0.36	0.76-0.12	1.3-28	0.46-1.0
DEF	<0.34	<0.074	-	-	-	-
EPN	0.44-3.4	0.13-1.0	-	-	4.1-7.9[a]	0.022-0.042[a]
Ethoprop	0.36-0.62	0.048-0.083	-	-	12-21	0.067-0.12
Methyl parathion	0.11-0.37	0.14-0.48	-	-	-	-
Phorate	0.09-0.21	0.29-0.68	-	-	0.24-0.41	0.18-0.32

[a] MATC and AF derived from entire life-cycle toxicity test.
[b] MATC and AF derived from partial life-cycle toxicity test.
[c] MATC and AF derived from embryo/juvenile toxicity test, unless noted.

Table 9. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to aldicarb. The mysids were 24- to 48-h-old at beginning of test. Twenty mysids were placed in each treatment (10 per replicate). Salinity was from 26-31 ⁰/oo and temperature 22±1°C.

Day	Control	Carrier control	Measured concentration (µg/ℓ)									
			0.38		0.50		1.0		1.5		2.1	
			A	B	A	B	A	B	A	B	A	B
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	10	0
5	0	0	0	0	0	0	0	0	0	0	10	0
6	0	0	0	0	0	0	0	0	0	0	10	0
7[a]	0	0	0	0	0	0	0	0	0	0	10	0
8	0	0	0	0	0	0	0	0	0	0	10	0
9	0	0	0	0	0	0	0	0	0	0	10	0
10	0	0	0	0	0	0	0	0	0	0	10	0
11	0	0	0	0	0	0	0	0	0	0	20	10
12	0	0	0	0	0	0	0	0	10	0	20	10
13	0	0	0	0	0	0	0	10	10	10	20	10
14[a]	0	0	0	0	0	0	0	10	10[b]	10[b]	20[b]	10[b]
15	0	0	0	0	0	0	0	10	10	10	20	10
16	0[c]	0[c]	0	0	0	0	0	10	10	10	20	10
17	0	0	0[c]	0[c]	0[c]	0[c]	0[c]	10[c]	10	10	20	10
18	0	0	0	10	0	10	0	10	10[c]	10	20	20
19	0	0	10	10	0	10	0	20	10	20[c]	20	20
20	0	0	10	10	10	10	0	20	10	30	20[c]	20
21[a]	0	0	10	10	10	10	0	20	10[b]	30[b]	20[b]	20[b,c]
22	0	0	10	20	10	20	0	20	10	30	20	20
23	0	0	10	20	10	20	0	20	10	30	20	30
24	0	0	10	20	10	20	0	20	10	30	20	30
25	0	0	10	20	10	20	0	20	20	40	20	30
26	10	0	10	20	10	20	0	20	30	40	20	40
27	10	0	10	20	10	20	0	20	30	40	40	40
28[a]	10	0	10	20	10	20	0	20	30[b]	40[b]	40[b]	40[b]
Averages:	10	0	15		15		10		35		40	

---

[a] Analysis of variance (ANOVA) performed.
[b] Significantly greater ($P \leq 0.05$) than the control as determined by ANOVA and Williams' test (Williams, 1971).
[c] Formation of brood pouches first observed.

Table 10. Number of offspring produced per female of mysid shrimp (*Mysidopsis bahia*) exposed to aldicarb in a chronic (28-day) exposure in natural, flowing seawater.

Measured concentration (μg/ℓ)	Replicate A	Replicate B	Total offspring	Females with brood pouches	Offspring per female
Control	24	-	24	4	6.0
Carrier control	28	-	28	4	7.0
0.38	26	22	48	7	6.8
0.50	16	40	56	9	6.2
1.0	38	29	67	9	7.4
1.5	10	9	19	4	4.8
2.1	0	0	0	3	0.0[a]

[a] Significantly less ($\alpha = 0.05$) than the control as determined by ANOVA and Williams' test (Williams, 1971).

Table 11. Survival of embryo and juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of aldicarb in seawater at 29±1°C. Juvenile fish were exposed for 28 days posthatch.

Exposure concentration (μg/ℓ)		Survival, %	
Nominal	Measured	Embryo	Juveniles
Control	ND[a]	92	86
Carrier control	ND	98	95
17	8.7	95	86
29	14	96	71
48	28	92	79
80	50	94	70
130	88	91	64

[a] ND = nondetectable (<0.2 μg/ℓ).

Table 12. Standard lengths and survival of juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of aldicarb for 28 days. One hundred embryos were placed in each treatment at the beginning of the test.

Exposure concentration (μg/ℓ)		Number of surviving fish	Average length (mm)
Nominal	Measured		
Control	ND[a]	69	11.5
Carrier control	ND	76	12.5
17	8.7	69	13.0
29	14	57	12.0
48	28	63	11.0
80	50	56	11.0
130	88	51	9.5[b]

[a] ND = nondetectable (<0.2 μg/ℓ)

[b] Fish significantly smaller ($\alpha = 0.05$) than the controls as determined by ANOVA and Williams' test (Williams, 1971).

Table 13. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to carbophenothion. The mysids were 24-h-old at the beginning of the test. Forty mysids were placed in each treatment. Salinity averaged 20.7 ^{0}/oo (17-23 ^{0}/oo); temperature, 25.2^{0}C (23.5 - 26.2^{0}C).

Day	Control	Carrier Control[a]	Measured Concentration (µg/ℓ)					
			0.18	0.30	0.48	1.2	1.9	4.1
1	2.5	0	7.5	0	2.5	7.5	0	2.5
2	2.5	0	7.5	2.5	5.0	7.5	0	2.5
3	5.0	2.9	7.5	2.5	7.5	7.5	0	7.5
4	5.0	2.9	12.5	2.5	7.5	7.5	0	17.5
5	5.0	2.9	12.5	5.0	7.5	7.5	0	20.0
6	5.0	5.7	12.5	7.5	7.5	7.5	0	20
7	5.0	5.7	12.5	7.5	7.5	7.5	0	20
8	5.0	5.7	12.5	7.5	7.5	7.5	0	22.5
9	7.5	5.7	12.5	7.5	7.5	7.5	0	22.5
10	10	5.7	12.5	7.5	7.5	7.5	0	27.5
11	10	11.4	12.5	10	7.5	10	0	27.5
12	10	14.3	12.5	10	7.5	10	5	27.5
13	10	14.3	12.5	10	10	10	7.5	27.5
14	10	14.3	12.5	12.5	10	12.5	7.5	30
15	10	14.3	15	17.5	10	12.5	7.5	30
16	20	14.3	15	22.5	12.5	15	15	35
17	20	17.1	20	22.5	15	17.5	17.5	35
18	20	17.1	20	25	15	17.5	17.5	37.5
19	20	20	20	35	15	17.5	22.5	37.5
20	20	22.9	20	40	15	20	22.5	37.5
21	20	22.9	20	40	15	22.5	25	37.5
22	20	22.9	20	40	15	27.5	25	37.5
23	20	22.9	20	42.5	15	27.5	27.5	37.5
24	20	25.7	20	42.5	15	27.5	27.5	37.5
25	20	25.7	20	42.5	15	27.5	27.5	37.5
26	20	25.7	20	42.5	15	27.5	27.5	42.5
27	25	28.6	22.5	42.5	15	32.5	27.5	42.5
28	30	28.6	22.5	42.5[b]	17.5	35	27.5	47.5[b]

[a] N = 35

[b] Significantly different than controls ($\alpha = 0.05$) at the end of test.

Table 14. Number of offspring per female of mysid shrimp (*Mysidopsis bahia*) exposed to carbophenothion in a chronic (28-day) exposure in natural, flowing seawater.

Measured concentration (μg/ℓ)	Total offspring	Offspring per female	Onset of spawning (day of test)
Control	104	34.2	19
Carrier control	78	25.5	19
0.18	102	35.6	20
0.30	110	47.6	18
0.48	121	42.6	20
1.2	112	47.9	20
1.9	84	31.5	20
4.1	52	16.8	22

Table 15. Survival of F_1 generation of mysids (Mysidopsis bahia) continuously exposed to carbophenothion. Values given are percentage survival 10 days posthatch.

Measured concentration ($\mu g/\ell$)	No. of trials	% survival	Range
Control	6	90	(80-100)
Carrier control	4	93.5	(80-100)
0.18	8	87.7	(50-100)
0.30	5	93.1	(80-100)
0.48	6	89.2	(75-100)
1.2	7	75.7	(20-100)
1.9	5	77.3[a]	(60-100)
4.1	1	33[a,b]	-

[a] Juvenile swimming behavior erratic, i.e., swimming in backward somersaults, frequent change of direction, very rapid swimming.

[b] Different from controls ($\alpha = 0.05$).

Table 16. Growth (head-tail length) of parental generation mysid shrimp (*Mysidopsis bahia*) exposed to carbophenothion in a chronic (28-day) exposure in natural, flowing seawater.

Measured concentration (μg/ℓ)	Number of shrimp measured	Average length (mm)	Range (mm)
Control	26	5.9	5.0-6.5
Carrier control	24	5.9	5.0-6.2
0.18	31	5.7	4.5-6.2
0.30	23	5.7	5.0-6.5
0.48	31	5.6	5.0-6.0
1.2	23	5.4[a]	4.8-6.0
1.9	22	5.2[a]	4.5-5.8
4.1	17	5.1[a]	4.0-5.8

[a] Significantly different from controls ($\alpha = 0.05$).

Table 17. Survival of adult grass shrimp (*Palaemonetes pugio*) continuously exposed to carbophenothion for 249 days in a partial life-cycle test. One hundred shrimp were placed in each treatment.

Exposure concentration (μg/ℓ)		Survival, %	
Nominal	Measured	Day 33	Day 249
Control	ND[a]	96	75
Carrier control	ND	97	68
0.125	0.06	94	79
0.25	0.22	98	64
0.50	0.36	91	52
1.0	0.69	97	45[b]
2.0	1.3	92	52
4.0	2.9	24[b]	0[b]

[a] ND = nondetectable (<0.2 μg/ℓ).

[b] Significantly different from controls ($\alpha = 0.05$).

Table 18. Effect of carbophenothion on fecundity of grass shrimp (*Palaemonetes pugio*). The three components of fecundity examined included total number of ovigerous females, average egg production and hatching success in each test concentration. The number of eggs produced per female was determined for at least 10 shrimp per test concentration. Using a separate group of shrimp, hatching success was determined for each test concentration. Since egg production is proportional to length of female, rostrum-telson length of each female was used as a covariate in all statistical analyses.

Measured concentration (µg/ℓ)	Total No. ovigerous females	Average egg production (eggs/♀)	Average female length (mm)	Average No. larvae hatched/♀	Average female length (mm)
Control	45	154.5	26.8	125.1	27.1
Carrier control	40	154.6	27.4	115.1	29.2
0.06	46	132.9	26.6	118.1	28.0
0.22	50	157.4	26.6	94.7	26.6
0.36	30[a]	154.1	26.9	142.8	30.9
0.69	29[a]	163.3	27.1	164.4	29.7
1.3	32[a]	156.0	27.1	120.2	29.4
2.9	6[a,b]	-[c]	-	125	28.5

[a] Significantly less than the controls by Day 147.
[b] Significantly less than the controls by Day 84.
[c] Egg production not determined for this concentration.

Table 19. Survival of F_1 generation grass shrimp (*Palaemonetes pugio*) larvae reared under conditions of continued exposure to carbophenothion. Each larval rearing trial was run with larvae produced by a single female.

Concentration (μg/ℓ)	Number of larval rearing trials	Average survival from hatching to Day 10, %	Range
Control	10	90	46-100
Carrier control	16	82	50-100
0.06	14	83	36-100
0.22	12	83	63-97
0.36	13	82	43-100
0.69	14	85	67-100
1.3	7	84	70-97
2.9	5	5[a]	0-26[a]

[a] Significantly different from survival of controls ($\alpha = 0.05$).

Table 20. Effect of carbophenothion on weight (g) of grass shrimp (*Palaemonetes pugio*) exposed in a partial life-cycle test. Weight determinations were made for all survivors in each test concentration at the end of the test (Day 249).

Measured concentration (μg/ℓ)	Average weight[a] (g)	N
Control	0.1771	75
Carrier control	0.1342	68
0.06	0.1555	79
0.22	0.1619	64
0.36	0.1510	52
0.69	0.1530	45
1.3	0.1222	52
2.9	-	-

[a] No significant difference ($\alpha = 0.05$) between control and exposed animals.

Table 21. Concentrations of carbophenothion (μg/g) whole body, wet weight in parental generation grass shrimp (*Palaemonetes pugio*), and bioconcentration factors (concentration measured in tissue divided by the average concentration in exposure water) at termination of a 249-day partial life-cycle toxicity test.

Measured water concentration (μg/ℓ)	Measured tissue concentration (μg/g)	Bioconcentration factor
Control	ND[a]	-
Carrier control	ND	-
0.06	ND	-
0.22	ND	-
0.36	0.14	390
0.69	0.36	520
1.3	0.47	360
		$\bar{x}$ = 420

[a] ND = nondetectable (<0.02 μg/g).

Table 23. Standard lengths, tissue concentrations, and bioconcentration factors (concentration measured in whole body, wet weight, divided by average measured water concentration) for juvenile sheepshead minnows (*Cyprinodon variegatus*) continuously exposed to various concentrations of carbophenothion in seawater at 30°C.

Exposure concentration (μg/ℓ)		Average standard length (mm)	Concentration in fish (μg/g)	Bioconcentration factor
Nominal	Measured			
Control	ND[a]	10.9	ND[a]	-
0.38	0.36	10.6	-	-
0.75	0.59	10.8	0.46	780
1.5	1.3	10.8	1.5	1,200
3.0	2.8	9.6[b]	2.4	860
6.0	5.4	-	-	-
12.	11.	-	-	-

[a] ND = nondetectable (<0.05 μg/ℓ water, <0.05 μg/g tissue).

[b] Significantly different from control and all lower concentrations.

Table 22. Survival of embryonic and juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of carbophenothion in seawater at 30°C.

Exposure concentration (µg/ℓ)		Survival (%)		
Nominal	Measured	Embryos	Juveniles	Both stages
Control	ND[a]	95	96	91
0.38	0.36	92	96	89
0.75	0.59	89	97	86
1.5	1.3	86	96	82
3.0	2.8	85	92	79[b]
6.0	5.4	86	4[c]	4[c]
12.	11.	86	0[c]	0[c]

[a] ND = nondetectable (<0.05 µg/ℓ).

[b] Significantly different from control and 0.36 µg/ℓ (α = 0.05).

[c] Significantly different from control and all other concentrations (α = 0.05).

Table 24. Animals collected from control aquaria and aquaria exposed to carbophenothion in eight-week benthic community study.

Taxa	Exposure concentration (μg/ℓ)				Total
Nominal Measured	Control (ND)[a]	0.01 (ND)	0.1 (0.1)	1.0 (0.9)	
Annelida					
Pectinariidae					
Cistenides gouldii	41	38	40	34	153
Nereidae					
Neathes succinea	25	19	8	20	72
Nereis pelagica	2	4	4	5	15
Laeonereis culveri	0	0	0	1	1
Perinereis floridana	0	0	1	0	1
Capitellidae					
Capitellides jonesi	1	2	0	1	4
Capitella capitata	1	1	0	5	7
Capitomastus aciculatus	0	2	0	0	2
Mediomastus californiensis	0	1	0	1	2
Spionidae					
Nerine agilis	0	1	0	0	1
Polydora ligni	8	4	1	1	14
Polydora websteri	5	9	1	1	16
Polydora socialis	1	0	1	0	2
Streblospio benedicti	6	2	0	3	11
Spiophanes bombyx	4	3	1	2	10
Sthenelais articulata	0	1	0	0	1
Sabellidae					
Sabella melanostigma	1	0	0	0	1
Phyllodocidae					
Nereiphylla fragilis	1	0	0	0	1
Eteone heteropoda	0	2	0	0	2
Eumida sanguinea	0	1	0	0	1
Opheliidae					
Armandia agilis	1	1	0	0	2
Orbiniidae					
Haploscoloplos robustus	0	0	0	1	1
Haploscoloplos fragilis	0	2	1	1	4

Table 24 continued.

Taxa		Exposure concentration (µg/ℓ)				Total
	Nominal	Control	0.01	0.1	1.0	
	Measured	(ND)[a]	(ND)	(0.1)	(0.9)	
Oweniidae						
Owenia fusiformis		0	0	0	2	2
Total Annelids		(97)	(93)	(58)	(78)	(326)
Nemertea						
Order Heteronemertea		1	0	0	0	1
Order Hoplonemertea		1	1	0	0	2
Order Paleonemertea		1	1	0	0	2
Platyhelminthes		0	1	2	1	4
Mollusca						
Mulinia lateralis		178	193	114	200	685
Ensis minor		51	43	27	44	165
Polinices duplicatus		0	2	1	0	3
Cyrtopleura costata		0	0	0	1	1
Musculus lateralis		0	0	0	1	1
Total Mollusca		(229)	(238)	(142)	(246)	(855)
Arthropoda						
Balanus improvisus		78	79	102	112	371
Anoplodactylus lentus		9	7	7	0	23
Rhithropanopeus harrisii		0	0	1	0	1
Corophium acherusicum		1	1	0	0	2
Family Caprellidae		0	1	0	0	1
Oxyurostylis smithi		0	0	1	0	1
Total Arthropoda		(88)	(88)	(111)	(112)	(399)
Coelenterata						
Aiptasia pallida		1	2	2	4	9
Chordata						
Molgula manhattensis		1	1	0	0	2
Total all phyla						
Individuals		419	425	315	441	1600
Species		23	29	18	21	41

[a] ND = nondetectable.

Table 25. Total number of individuals and species (in parentheses), by phylum, collected in an eight-week benthic community test with carbophenothion.

		Exposure concentration (µg/ℓ)				
Phylum	Nominal Measured	Control (ND)[a]	0.01 (ND)	0.1 (0.1)	1.0 (0.9)	Total
Mollusca		229	238	142	246	855
		(2)	(3)	(3)	(4)	(5)
Arthropoda		88	88	111	112	399
		(3)	(4)	(4)	(1)	(6)
Annelida		97	93	58	78	326
		(13)	(17)	(9)	(14)	(24)
Coelenterata		1	2	2	4	9
		(1)	(1)	(1)	(1)	(1)
Nemertea		3	2	0	0	5
		(3)	(2)	(0)	(0)	(3)
Platyhelminthes		0	1	2	1	4
		(0)	(1)	(1)	(1)	(1)
Chordata		1	1	0	0	2
		(1)	(1)	(0)	(0)	(1)
Total all phyla						
Individuals		419	425	315	441	1,600
Species		(23)	(29)	(18)	(21)	(41)

[a] ND = nondetectable.

Table 26. Octanol/water partition coefficients (Log P) of methyl parathion, phorate, EPN, carbophenothion, and DEF, as determined by reversed phase high pressure liquid chromatography.

Chemical	Log P
Benzene[a]	2.1
Methyl parathion[b]	2.4
Phorate[c]	3.2
Biphenyl[a]	3.8
EPN[d]	4.0
Carbophenothion[e]	4.9
DEF[f]	5.7
p,*p*-DDE[a]	5.7
Hexachlorobenzene[a]	6.2

[a] Reference chemical for calibration.
[b] *O*,*O*-dimethyl-*O*-*p*-nitrophenyl phosphorothioate.
[c] *O*,*O*-diethyl-*S*-(ethyl,thio) methyl phosphorodithioate.
[d] *O*,ethyl-*O*-*p*-nitrophenyl phosphorothioate.
[e] *S*-[(p-chlorophenyl thio)methyl]*O*,*O*-diethyl phosphorodithioate.
[f] *S*,*S*,*S*-tributyl phosphorotrithioate.

Table 27. Results of persistence studies with carbophenothion. Numbers represent micrograms (μg) of the pesticide detected in the respective compartment. The totals are the percentages of the combined micrograms of pesticide detected in a system when compared to the micrograms originally added to the system. ND means no pesticide detected.

System	Compartment	Time		
		Day 0	Day 5	Day 7
Untreated[a]	water (μg)	4.9	ND	
	sediment (μg)	3.8	5.7	
	total (%)	87%	57%	
Sterile[b]	water (μg)			ND
	sediment (μg)			6.1
	total (%)			61%
Seawater[c]	water (μg)	9.5		ND
	resin (μg)	ND		4.1
	total (%)	95%		41%

[a] Ten grams of sediment (wet weight) and 100 mℓ of 20 μm filtered seawater of 27 parts per thousand salinity fortified with 10 μg of carbophenothion in 10 μℓ of acetone so that at Day 0, water concentration equivalent to 49 μg/ℓ and sediment concentration equivalent to 3800 μg/kg (dry weight). Systems aerated at midpoint of water column at 50 mℓ per minute flow rate, with pesticide in exiting air purged through XAD-4 resin trap.

[b] Same as untreated systems except that 5 mℓ of formalin was added 24 hours before Day 0. Sterility was confirmed by plating aliquots from the water column on 15 parts per thousand Zobell's media throughout the test.

[c] Same as untreated systems, except that sediment was not added.

Table 28. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to DEF. The mysids were 24-h-old at the beginning of the test. Forty mysids were placed in each treatment unless otherwise noted. Salinity was from 24-32 ^{o}/oo and temperature, 22-27°C.

Day	Control (N = 36)	Carrier control	Measured concentration (µg/ℓ)			
			0.34	0.68 (N = 39)	1.7	3.3
1	0	0	0	0	0	5
2	0	0	0	0	0	5
3	0	0	0	0	0	5
4	2.8	0	0	0	5.0	57.5
5	2.8	0	0	0	7.5	82.5
6	2.8	0	0	0	7.5	82.5
7	5.6	0	0	7.7	17.5	92.5
8	5.6	0	0	7.7	20.0	97.5
9	5.6	0	0	7.7	20.0	100.0
10	5.6	0	0	7.7	20.0	100.0
11	5.6	0	0	10.2	45.0	100.0
12	5.6	0	0	10.2	45.0	100.0
13	8.3	0	0	12.8	45.0	100.0
14	8.3	0	0	17.9	47.5	100.0
15	8.3	0	0	17.9	50.0	100.0
16	11.1	2.5	0	17.9	55.0	100.0
17	14.9	2.5	0	20.5	55.0	100.0
18	14.9	5.0	0	23.1	55.0	100.0
19	14.9	5.0	0	28.2	57.5	100.0
20	14.9	10.0	0	30.1	57.5	100.0
21	19.4	12.5	7.5	35.9	60.0	100.0
22	22.2	12.5	7.5	41.0	62.5	100.0
23	22.2	12.5	7.5	48.7	65.0	100.0
24	25.0	17.5	7.5	51.3	72.5	100.0
25	25.0	17.5	10.0	51.3	75.5	100.0
26	30.1	17.5	10.0	51.3	77.5	100.0
27	30.1	17.5	12.5	56.4	80.0	100.0
28	30.1	22.5	15.0	58.9[a]	80.0[a]	100.0[a]

---

[a] Significantly different from controls (α = 0.05).

Table 29. Number of offspring per female mysid shrimp (Mysidopsis bahia) exposed to DEF in a 28-day life-cycle toxicity test.

Concentration (µg/ℓ) Nominal	Measured	Total offspring	Offspring per female	(range)
Control	ND[a]	362	22.6	(13.7-42.0)
Carrier control	ND	413	20.5	(5.3-28.0)
0.5	0.34	254	10.8[b]	(7.0-16.0)
1.0	0.68	88	6.6[b]	(0.0-10.0)
2.0	1.7	0	0[b]	

[a] ND = nondetectable (<0.02 µg/ℓ).

[b] Significantly different from controls at (α = .05).

Table 30. Results of persistence studies with DEF. Numbers represent micrograms (μg) of the pesticide detected in the respective compartment. The totals are the percentages of the combined micrograms of pesticide detected in a system when compared to the micrograms originally added to the system. ND means no pesticide detected.

System	Compartment	Time		
		Day 0	Day 5	Day 7
Untreated[a]	water (μg)	7.5	ND	
	sediment (μg)	3.1	1.9	
	total (%)	106%	19%	
Sterile[b]	water (μg)			ND
	sediment (μg)			7.7
	total (%)			77%
Seawater[c]	water (μg)	9.8		3.8
	resin (μg)	ND		1.2
	total (%)	98%		50%

[a] Ten grams of sediment (wet weight) and 100 mℓ of 20 μm filtered seawater with 27 parts per thousand salinity fortified with 10 μg of DEF in 10 μℓ of acetone so that at Day 0, water concentration equivalent to 75 μg/ℓ and sediment concentration equivalent to 3100 μg/kg (dry weight). Systems aerated at midpoint of water column at 50 mℓ per minute flow rate, with pesticide in exiting air purged through XAD-4 resin trap.

[b] Same as untreated systems except that 5 mℓ of formalin was added 24 hours before Day 0. Sterility was confirmed by plating aliquots from the water column on 15 parts per thousand Zobell's media throughout the test.

[c] Same as untreated systems, except that sediment was not added.

Table 31. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to EPN. The mysids were 24-h-old at the beginning of the test. Forty mysids were placed in each treatment. Salinity was from 24-32 $^{0}/oo$ and temperature, 21-24^{o}C.

Day	Control	Carrier control	Measured concentration (µg/ℓ) 0.06	0.08	0.44	4.1
1	0	0	0	0	0	0
2	2.5	0	0	2.5	0	0
3	2.5	2.5	0	2.5	0	2.5
4	2.5	2.5	0	2.5	0	5.0
5	2.5	2.5	0	2.5	0	5.0
6	2.5	2.5	0	2.5	0	5.0
7	2.5	2.5	0	2.5	0	5.0
8	2.5	2.5	0	2.5	0	5.0
9	2.5	2.5	0	2.5	0	5.0
10	2.5	2.5	0	2.5	0	5.0
11	2.5	2.5	0	2.5	0	7.5
12	2.5	2.5	0	5.0	0	10.0
13	2.5	2.5	0	5.0	5.0	15.0
14	2.5	2.5	0	5.0	5.0	20.0
15	2.5	2.5	0	5.0	7.5	20.0
16	2.5	2.5	0	5.0	7.5	20.0
17	2.5	2.5	0	5.0	7.5	22.5
18	2.5	2.5	0	5.0	7.5	25.5
19	2.5	2.5	0	5.0	7.5	30.0
20	2.5	2.5	0	5.0	7.5	35.0
21	2.5	2.5	0	5.0	7.5	37.5
22	5.0	2.5	0	5.0	7.5	47.5
23	5.0	2.5	0	7.5	7.5	57.5
24	7.5	2.5	0	7.5	7.5	65.0
25	7.5	2.5	0	7.5	7.5	65.0
26	7.5	2.5	0	7.5	7.5	65.0
27	7.5	2.5	0	7.5	7.5	65.0
28	7.5	2.5	0	7.5	7.5	67.5[a]

[a] Significantly different from controls ($\alpha = 0.05$) at the end of test.

Table 32. Number of offspring per female mysid shrimp (*Mysidopsis bahia*) exposed to EPN in a chronic (28-day) exposure in natural, flowing seawater.

Concentration (μg/ℓ) Nominal	 Measured	Total offspring	Offspring per female (range)
Control	ND[a]	306	16.1 (10.0-21.0)
Carrier control	ND	219	14.6 (6.0-31.5)
0.075	0.06	426	18.5 (10.0-32.5)
0.10	0.08	206	14.8 (9.0-23.0)
0.30	0.44	301	15.9 (9.5-25.5)
3.0	4.1	7	3.5[b] (1.0-6.0)

[a] ND = nondetectable (<0.02 μg/ℓ).
[b] Significantly different from controls ($\alpha = 0.05$).

Table 33. Percentage survival of sheepshead minnows (*Cyprinodon variegatus*) continuously exposed to EPN for 265 days in an entire life-cycle toxicity test. The exposure began with 118 to 121 embryos per treatment. The survival data are divided into: (1) percentage of embryos that hatched; (2) percentage of hatched fish that survived as juveniles until the 28th day of the test; and (3) percentage of adult fish that survived from Day 28, when the total number of fish was reduced to 60 per treatment, until Day 140. Since there were no significant differences, duplicates were combined.

Measured concentration (μg/ℓ)	Percentage Survival			
	Embryos to Hatching	Hatching to Day 28	Day 28 to 140	Total[a]
Control, ND[b]	90.0	99.1	96.8	86.3
0.25	90.0	99.2	100.0	89.3
0.50	88.8	96.1	96.6	82.4
0.88	94.1	97.4	98.3	90.1
2.2	83.4	94.9	93.3	73.8
4.1	89.2	95.9	85.7	73.3
7.9	83.3	96.7	83.0[c]	66.9

[a] Product of survival percentages for embryos to hatching, hatching to Day 28, and Day 28 to 140. Statistical analyses were not performed on data in the "Total" column.

[b] Carrier control, ND = nondetectable (<0.05 μg/ℓ).

[c] Significantly different from survival of controls, (Chi-square test, $\alpha = 0.05$).

Table 34. Effect of EPN on average standard length (mm) of sheepshead minnows (*Cyprinodon variegatus*) exposed for an entire life-cycle.

Measured concentration (µg/ℓ)	Day of exposure				
	28[a]	60[a]	88[a]	118	140
ND; Control[b]	10.6	19.7	24.6	29.4	32.4
0.25	10.0[c]	19.6	24.5	29.7	32.0
0.50	9.9[c]	19.3	24.2	29.0	32.1
0.88	10.0[c]	19.5	24.0	28.1	32.2
2.2	9.7[c]	18.5[c]	23.6	28.1	31.8
4.1	9.6[c]	18.8	23.4	28.5	31.6
7.9	9.7[c]	17.1[c]	20.7[c]	25.9[c]	28.8[c]

[a] Location was significant in ANOVA ($\alpha < 0.05$) but there was no significant location x concentration interaction; therefore, duplicates were combined for these analyses.

[b] Control with carrier; ND = nondetectable (<0.05 µg/ℓ).

[c] Standard lengths differed from those of control fish; Analysis of Variance and Duncan's Multiple range test ($\alpha = 0.05$).

Table 35. Egg production by sheepshead minnows (Cyprinodon variegatus) continuously exposed to measured concentrations of EPN in flowing seawater at 30°C. Spawning groups usually consisted of two males and three females from a given concentration. Percentage fertility is in parentheses.

Exposure concentration (μg/ℓ)	Duplicate	Average number eggs/female/day									
		Trial I[a]		Trial II[b]		Trial III[a]		Duplicate $\bar{X}$		All Trials $\bar{X}$	
ND[c]	upper	12.8	(99.0)	13.2	(98.7)	13.8	(96.9)	13.3	(98.2)	7.9	(98.1)
ND	lower	7.5	(96.0)	0.0	(100[d])	0.0[e]	-	2.5	(98.0)		
0.25	upper	18.2[f]	(100)	7.8	(99.4)	5.0	(98.0)	10.3	(99.1)	13.1	(97.9)
0.25	lower	17.6	(98.9)	0.4	(94.1)	29.8[e]	(97.3)	15.9	(96.8)		
0.50	upper	23.0[e]	(99.8)	20.1	(99.8)	9.1	(99.3)	17.4	(99.6)	11.8	(99.6)
0.50	lower	17.5	(99.0)	1.0	(100)	0.0	(100[d])	6.2	(99.7)		
0.88	upper	16.2	(99.0)	2.4	(100)	17.6	(99.4)	12.1	(99.5)	8.6	(99.4)
0.88	lower	15.2	(99.3)	0.0	-	0.0[e]	-	5.0	(99.3)		
2.2	upper	12.3	(100)	0.0	-	0.0	-	4.1	(100)	3.8	(99.2)
2.2	lower	10.7	(98.4)	0.0	-	0.0	-	3.6	(98.4)		
4.1	upper	9.0[e]	(97.2)	0.0	-	0.0[e]	-	3.0	(97.2)	2.6	(98.6)
4.1	lower	3.9	(100)	0.0	-			2.0	(100)		
7.9	upper	0.1	(100)	0.5	(100)	0.1	(100)	0.2	(100)	0.5	(93.8)
7.9	lower	1.7	(75.0)	0.0	-			0.9	(75.0[g,h])		

[a] Spawning data for 10 days were analyzed.
[b] Spawning data for 14 days were analyzed.
[c] Carrier control, ND = nondetectable (<0.05 μg/ℓ).
[d] Although less than 0.1 eggs/female/day were produced all were fertile.
[e] Two females and three males.
[f] One female and four males.
[g] Significantly different from all other treatments ($\alpha = 0.05$).
[h] Significant ($\alpha = 0.05$) duplicate x concentration interaction.

Table 36. Average dissolved oxygen concentrations (mg/ℓ) during a 265-day toxicity test in which sheepshead minnows (*Cyprinodon variegatus*) were continuously exposed to measured concentrations of EPN at 30°C. Dissolved oxygen content was determined weekly in one duplicate exposure aquarium in each exposure concentration.

Day	Portion of test	N	Mean	Standard deviation
0-28	Parental embryo/ juvenile survival	28	4.9[a]	0.64
29-149	Juvenile and adult survival	119	3.5	0.76
150-161	Spawning trial I	14	2.5[a]	0.80
192-209	Spawning trial II	21	3.6	0.84
216-231	Spawning trial III	14	3.2	0.87
150-231	All spawning trials	84	3.5	0.97
222-259	Progeny survival	35	3.3	0.86
0-265	Entire test	266	3.6	0.94

[a] Significantly different from other portions of the test; $\alpha = 0.05$.

Table 37. Survival of embryos, fry, and juvenile sheepshead minnows (*Cyprinodon variegatus*) and standard lengths of surviving fish continuously exposed to measured concentrations of EPN for 28 days. Exposure began with 80 embryos per duplicate aquarium. Embryos were spawned by adult fish that had been continuously exposed in the respective concentrations for greater than 222 days. Since no significant difference ($\alpha = 0.05$) existed between treatments, duplicates have been combined.

Exposure concentration (μg/ℓ)	Survival (%)			Average standard length (mm)
	Embryos	Fry and juveniles	Both stages	
ND[a]	81.2	93.2	75.0	8.9
0.25	90.0[b]	100.0	90.0	9.0
0.50	90.1[b]	98.7	88.9	8.8
0.88	91.7[c]	92.7	85.0	8.6

[a] Carrier control, ND = nondetectable (<0.05 μg/ℓ).
[b] 81 embryos.
[c] 120 embryos.

Table 38. Concentrations of EPN (µg/g, whole body, wet weight) measured in selected life stages of the sheepshead minnow (*Cyprinodon variegatus*) in a 265-day life-cycle toxicity test.

Life stage, Days exposed	Average measured exposure concentration, (µg/ℓ)						
	Control, ND[a]	0.25	0.50	0.88	2.2	4.1	7.9
Juveniles, 28	ND[a]	ND	ND	0.40	2.3	6.4	8.0
Adult males, 161	ND	0.31	1.0	1.8	44.	3.9	67.
Adult females, 161	ND	0.21	0.64	2.9	15.	38.	81.
Adult fish, 235	ND	0.39	1.0	4.3	5.4	21.	46.
Embryos, 146 to 200	ND	ND	0.60	1.9	1.5		
Juvenile progeny, 28	ND	ND	0.29	0.63			

[a] ND = nondetectable (<0.05 µg/ℓ water, <0.25 µg/g tissues).

Table 39. Bioconcentration factors (concentration measured in tissue divided by the average concentration measured in exposure water) for EPN in selected life-stages of the sheepshead minnow (*Cyprinodon variegatus*) in a 265-day life-cycle toxicity test. EPN was not detected in control fish or embryos.

Life stage, Days exposed	Exposure concentration (μg/ℓ)					
	0.25	0.50	0.88	2.2	4.1	7.9
Juveniles, Day 28	ND[a]	ND	450	1,000	1,600	1,000
Adult males, Day 161	1,200	2,000	2,000	20,000	950	8,500
Adult females, Day 161	840	1,300	3,300	6,800	9,300	10,000
Adult fish, Day 235	1,600	2,000	4,900	2,500	5,100	5,800
Embryos, Days 146 to 200	ND	1,200	2,200	680		
Juvenile progeny, Day 28	ND	580	720			

[a] ND = nondetectable EPN in tissues (<0.25 μg/g); therefore, no bioconcentration factor could be calculated.

Table 40. Acetylcholinesterase activity, expressed as micromoles of acetylcholine hydrolyzed/hour/mg brain tissue x 100, in brains from sheepshead minnows (Cyprinodon variegatus) that were continuously exposed to EPN. Each assay sample consisted of pooled brains from three fish; three samples were assayed to obtain each datum.

Measured concentration (µg/ℓ)	Day 161 AChE	Day 161 % Inhibition	Day 235 AChE	Day 235 % Inhibition
ND[a]	177	-	205	-
0.25	117[b]	34	118[b]	42
0.50	81[b]	54	81[b]	60
0.88	51[b]	71	58[b]	72
2.2	38[b]	78	35[b]	83
4.1	32[b]	82	36[b]	82
7.9	24[b]	86	30[b]	85

[a] Carrier control, ND = nondetectable (<0.05 µg/ℓ).
[b] Significantly less than that of controls ($\alpha = 0.05$).

Table 41. Results of persistence studies with EPN. Numbers represent micrograms (μg) of the pesticide detected in the respective compartment. The totals are the percentages of the combined micrograms of pesticide detected in a system when compared to the micrograms originally added to the system. ND means no pesticide detected.

System	Compartment	Time		
		Day 0	Day 5	Day 7
Untreated[a]	water (μg)	6.2	ND	
	sediment (μg)	2.1	2.8	
	total (%)	83%	28%	
Sterile[b]	water (μg)			ND
	sediment (μg)			7.9
	total (%)			79%
Seawater[c]	water (μg)	9.7		3.9
	resin (μg)	ND		ND
	total (%)	97%		39%

[a] Ten grams of sediment (wet weight) and 100 mℓ of 20 μm filtered seawater with 27 parts per thousand salinity fortified with 10 μg of EPN in 10 μℓ of acetone so that at Day 0, water concentration was equivalent to 62 μg/ℓ and sediment concentration equivalent to 2100 μg/kg (dry weight). Systems aerated at midpoint of water column at 50 mℓ per minute flow rate, with pesticide in exiting air purged through XAD-4 resin trap.

[b] Same as untreated systems except that 5 mℓ of formalin was added 24 hours before Day 0. Sterility was confirmed by plating aliquots from the water column on 15 parts per thousand Zobell's media throughout the test.

[c] Same as untreated systems, except that sediment was not added.

Table 42. Mortality (percentage killed) of mysids (*Mysidopsis bahia*) during a chronic (28-day) exposure to ethoprop. The mysids were 24- to 48-h old at the beginning of the test. Twenty mysids were placed in each ethoprop treatment (ten per replicate); ten mysids in the control and solvent control treatments. Salinity was from 26 to 31 $^0/oo$ and temperature, 22 ± 1^0C.

			Measured concentration (µg/ℓ)									
		Carrier	0.15		0.27		0.36		0.62		1.4	
Day	Control	control	A	B	A	B	A	B	A	B	A	B
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	10	0	0	0
7[a]	0	0	0	0	0	0	10	0	20	0	0	0
8	0	0	0	0	0	0	10	0	20	0	0	0
9	0	0	0	0	0	0	10	0	20	0	0	0
10	0	0	0	0	0	0	10	0	20	0	0	0
11	0	0	0	0	0	0	10	0	20	0	0	0
12	0	0	0	0	0	0	10	0	20	0	0	0
13	0	0	0	0	0	0	10	0	20	0	0	10
14[a]	0	0	0	0	0	0	10	0	20	0	0	10
15	0	0	0	0	0	0	10	0	20	0	0	10
16	0	0[b]	0	0[b]	0	0[b]	10	0	20	0	0	10
17	0[b]	0	0[b]	0	0[b]	0	10[b]	0[b]	20	0	0	10
18	0	0	0	0	0	0	10	0	20[b]	0	0[b]	10
19	0	0	0	0	0	0	10	0	20	0[b]	0	10[b]
20	0	0	0	0	0	0	10	0	20	0	10	10
21[a]	0	0	0	0	0	0	10	0	20[c]	10[c]	20[c]	20[c]
22	0	0	0	0	0	0	10	0	20	10	30	20
23	0	0	0	0	0	0	10	0	20	10	30	20
24	0	0	10	0	0	0	10	0	20	20	30	30
25	0	0	10	10	10	0	10	0	20	20	30	40
26	0	0	10	10	10	0	10	0	20	20	40	40
27	0	10	10	10	10	0	10	0	20	20	50	40
28[a]	0	10	10	10	10	0	10	0	20[c]	20[c]	50[c]	40[c]
Average:	0	10	10		5		5		20[c]		45[c]	

[a] Statistical analysis performed.

[b] Formation of brood pouches first observed.

[c] Significantly greater (α = 0.05) than the control.

Table 43. Number of offspring produced per female of mysid shrimp (*Mysidopsis bahia*) exposed to ethoprop in a chronic (28-day) exposure in natural, flowing seawater.

Measured concentration (µg/ℓ)	Number of offspring Rep A	Rep B	Total	Females with brood pouches	Offspring per female
Control, ND[a]	38	-	38	6	6.3
Carrier control	38	-	38	6	6.3
0.15	41	35	76	11	6.9
0.27	30	36	66	11	6.0
0.36	26	11	37	6	6.2
0.62	22	16	38	7	5.4
1.4	11	5	16	4	4.0[b]

[a] ND = nondetectable (<0.2 µg/ℓ).

[b] Significantly less ($\alpha = 0.05$) than the control.

Table 44. Survival of embryo and juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of ethoprop in seawater at 30±1°C. Juvenile fish were exposed for 28 days posthatch.

Exposure concentration (μg/ℓ)		Survival, %	
Nominal	Measured	Embryo	Juveniles
Control	ND[a]	95	74
Carrier control	ND	96	78
26	12	94	68
43	21	89	45[b]
72	35	95	11[b]
120	54	91	5[b]
200	98	90	2.5[b]

[a] ND = nondetectable (<0.5 μg/ℓ).
[b] Significantly different from control (α = 0.05).

Table 45. Standard lengths, tissue concentrations, and bioconcentration factors of ethoprop in juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of ethoprop for 28 days.

Concentration (μg/ℓ) Nominal	Concentration (μg/ℓ) Measured	Concentration in fish (μg/g)	Average length (mm)	Bioconcentration factor
Control	ND[a]	ND	12	-
Carrier control	ND	ND	14	-
26	12	0.20	12	17
43	21	0.10	13	5
72	35	0.36	-[b]	10
120	54	0.48	-[b]	9
200	98	0.42	-[b]	4

[a] ND = nondetectable (<0.05 μg/ℓ water, <0.09 μg/g tissue).

[b] Length not measured because of small number of surviving fish.

Table 46. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (24-day) exposure of methyl parathion. The mysids were 24-h-old at the beginning of the test. Forty mysids were placed in each treatment unless otherwise noted. Salinity was from 12-25 $^{0}/oo$ and temperature, 24-27°C.

Day	Control (N = 35)	Carrier control (N = 35)	Measured concentration (µg/ℓ)			
			0.05	0.11	0.37	0.59
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	12.5
4	0	0	0	0	2.5	17.5
5	0	0	2.5	2.5	2.5	20.0
6	0	0	2.5	2.5	2.5	30.0
7	0	0	5.0	2.5	5.0	35.0
8	0	0	7.5	2.5	10.0	37.5
9	3.0	3.0	7.5	10.0	17.5	55.0
10	3.0	11.0	10.0	15.0	27.5	65.0
11	3.0	17.0	10.0	15.0	37.5	80.0
12	3.0	17.0	10.0	15.0	42.5	82.5
13	3.0	20.0	10.0	25.0	50.0	82.5
14	3.0	20.0	10.0	25.0	60.0	85.0
15	3.0	23.0	10.0	25.0	62.5	92.5
16	3.0	23.0	10.0	30.0	75.0	97.5
17	3.0	23.0	10.0	32.5	77.5	97.5
18	3.0	29.0	15.0	37.5	80.0	97.5
19	6.0	34.0	15.0	37.5	80.0	100.0
20	6.0	34.0	15.0	40.0	82.5	100.0
21	6.0	34.0	15.0	42.5	85.0	100.0
22	9.0	34.0	15.0	42.5	85.0	100.0
23	9.0	37.0	15.0	42.5	87.5	100.0
24	9.0	43.0	15.0	50.0	87.5[a]	100.0[a]

[a] Significantly different from controls (α = 0.05) at the end of test.

Table 47. Number of offspring per female mysid shrimp (*Mysidopsis bahia*) exposed to methyl parathion in a chronic (24-day) exposure in natural, flowing seawater.

Concentration (μg/ℓ) Nominal	Concentration (μg/ℓ) Measured	Total offspring	Offspring per female (range)
Control	ND[a]	183	8.3 (5.0-15.7)
Carrier control	ND	53	5.9 (3.5-11.0)
0.095	0.05	206	11.4 (3.3-20.3)
0.18	0.11	139	11.6 (2.0-15.0)
0.38	0.37	0[b]	0[b]
0.75	0.59	0[b]	0[b]

[a] ND = nondetectable; <0.02 μg/ℓ in seawater.
[b] Significantly different from controls at $\alpha = 0.05$.

Table 48. Results of persistence studies with methyl parathion. Numbers represent micrograms (μg) of the pesticide detected in the respective compartment. The totals are the percentages of the combined micrograms of pesticide detected in a system when compared to the micrograms originally added to the system. ND means no pesticide detected.

System	Compartment	Time		
		Day 0	Day 5	Day 7
Untreated[a]	water (μg)	8.8	0.11	ND
	sediment (μg)	0.9	0.19	ND
	total (%)	97%	3%	
Sterile[b]	water (μg)			6.5
	sediment (μg)			3.9
	total (%)			104%
Seawater[c]	water (μg)	9.9		7.5
	resin (μg)	ND		ND
	total (%)	99%		75%

		Day 0	Day 3	Day 6
Sunlight[d]	light (μg)	8.8	6.6	3.8
	total	100%	75%	43%
	dark (μg)	8.8	7.5	6.5
	total	100%	85%	73%

[a] Ten grams of sediment (wet weight) and 100 mℓ of filtered seawater (20 μm) with 27 parts per thousand salinity were fortified with 10 μg methyl parathion in 10 μℓ of acetone, so that at Day 0, water concentration of 88 μg/ℓ and sediment concentration of 900 μg/kg (dry weight). Systems were aerated at midpoint of water column at 50 mℓ per minute flow rate; pesticide in exiting air was sorbed by a XAD-4 resin trap.

[b] As in untreated systems except that 5 mℓ of formalin was added 24 hours before Day 0. Sterility was confirmed by plating aliquots from the water column on 15 parts per thousand Zobell's media throughout the study.

[c] Same as untreated systems, except that sediment was not added.

[d] Systems were 250 mℓ Pyrex Erlenmeyer flasks fitted with ground glass stoppers. A homogeneous solution in filtered (20 μm) seawater of 27 parts per thousand was prepared by adding 350 μg of methyl parathion in acetone to a 4-ℓ amber bottle, purging the acetone with a stream of nitrogen, and adding 3500 mℓ seawater. The bottle was shaken overnight and 100 mℓ aliquot was added to each flask. The flasks were stoppered and half were covered with foil. The systems were exposed outdoors to direct sunlight, where temperatures in the flasks varied from a high of 46°C during the day to a low of 20°C at night.

Table 49. Mortality (percentage killed) of mysid shrimp (*Mysidopsis bahia*) during a chronic (28-day) exposure to phorate. The mysids were 24-to 48-h-old at the beginning of the test. Forty mysids were placed in each treatment unless otherwise noted. Salinity was from 20-26 $^{o}/oo$ and temperature, 26-29°C.

			Measured concentration (µg/ℓ)			
Day	Control	Carrier control	0.02 (N = 35)	0.06	0.09 (N = 39)	0.21 (N = 38)
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	2.5	3.0	0	0	0
5	0	2.5	3.0	0	0	0
6	2.5	2.5	3.0	5.0	5.0	24.0
7	2.5	2.5	9.0	5.0	5.0	24.0
8	5.0	2.5	9.0	5.0	20.0	66.0
9	5.0	2.5	9.0	5.0	20.0	66.0
10	7.5	10.0	9.0	5.0	20.0	74.0
11	7.5	10.0	9.0	5.0	20.0	74.0
12	7.5	12.5	9.0	5.0	20.0	74.0
13	10.0	15.0	9.0	7.5	20.0	74.0
14	12.5	15.0	14.0	10.5	26.0	74.0
15	12.5	15.0	14.0	12.5	26.0	74.0
16	12.5	15.0	14.0	12.5	26.0	74.0
17	15.0	15.0	20.0	12.5	26.0	76.0
18	15.0	15.0	20.0	12.5	28.0	76.0
19	17.5	15.0	20.0	12.5	28.0	76.0
20	17.5	15.0	20.0	12.5	31.0	79.0
21	17.5	20.0	26.0	12.5	31.0	82.0
22	17.5	25.0	26.0	12.5	33.0	82.0
23	20.0	27.5	26.0	15.0	38.0	82.0
24	30.0	30.0	29.0	20.0	41.0	82.0
25	30.0	32.5	34.0	22.5	46.0	82.0
26	37.5	32.5	37.0	35.0	51.0	82.0
27	37.5	32.5	43.0	35.0	53.0	82.0
28	37.5	40.0	43.0	37.5	53.0	82.0[a]

[a] Significantly different from controls ($\alpha = 0.05$) at the end of test.

Table 50. Number of offspring per female mysid shrimp (*Mysidopsis bahia*) exposed to phorate in a chronic (28-day) exposure in natural, flowing seawater.

Concentration (μg/ℓ)		Total offspring	Offspring per female (range)
Nominal	Measured		
Control	ND[a]	292	16.8 (3.0-25.5)
Carrier control	ND	234	12.5 (6.0-21.0)
0.03	0.02	155	9.2 (2.0-13.7)
0.08	0.06	91	6.8 (2.0-12.0)
0.16	0.09	141	12.8 (2.0-23.0)
0.32	0.21	19	8.2 (2.5-14.0)

[a] ND = Nondetectable (<0.02 μg/ℓ).

Table 51. Survival of embryonic and juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of phorate in seawater for 28 days at 30°C.

Exposure concentration (μg/ℓ)		Survival (%)		
Nominal	Measured	Embryos	Juveniles	Both stages
Control	ND[a]	97.5	98.7	96.2
0.09	0.16	92.5	97.3	90.0
0.19	0.24	95.0	97.4	92.5
0.38	0.41	98.8	88.6[c]	87.5
0.75	0.77	96.2	57.1[b]	55.0[b]
1.5	1.2	97.5	3.8[b]	3.8[b]
3.0	2.5	88.8	0[b]	0[b]

[a] ND = nondetectable (<0.02 μg/ℓ).
[b] Significantly different from control ($\alpha = 0.01$).
[c] Significantly different from control ($\alpha = 0.05$).

Table 52. Standard lengths, tissue concentrations, and bioconcentration factors of phorate in juvenile sheepshead minnows (*Cyprinodon variegatus*) exposed to various concentrations of phorate for 28 days.

Concentration (μg/ℓ)		Concentration in fish (μg/g)	Average length (mm)	Bioconcentration factor
Nominal	Measured			
Control	ND[a]	ND	9.5	-
0.094	0.16	ND	9.3	-
0.19	0.24	ND	9.3	-
0.38	0.41	ND	8.9	-
0.75	0.77	0.069	8.8	90
1.5	1.2	-	10.7	-

[a] ND = nondetectable (<0.02 μg/ℓ water, <0.02 μg/g tissue).

Table 53. Results of persistence studies with phorate. Numbers represent micrograms (µg) of the pesticide detected in the respective compartment. The totals are the percentages of the combined micrograms of pesticide detected in a system when compared to the micrograms originally added to the system. ND means no pesticide detected.

System	Compartment	Time		
		Day 0	Day 5	Day 7
Untreated[a]	water (µg)	6.5	ND	
	sediment (µg)	1.9	ND	
	total (%)	84%		
Sterile[b]	water (µg)			ND
	sediment (µg)			ND
	total (%)			
Seawater[c]	water (µg)	9.7		ND
	resin (µg)	ND		3.1
	total (%)	97%		31%

[a] Ten grams of sediment (wet weight) and 100 mℓ of filtered seawater (20 µm) of 27 parts per thousand salinity fortified with 10 µg of phorate in 10 µℓ of acetone, so that at Day 0, water concentration of 65 µg/ℓ and sediment concentration of 1,900 µg/kg (dry weight). Systems were aerated at midpoint of water column at 50 mℓ per minute flow rate; pesticide in exiting air was sorbed by a XAD-4 resin trap.

[b] Same as untreated systems except that 5 mℓ of formalin was added 24 h prior to Day 0. Sterility was confirmed by plating aliquots from the water column on 15 parts per thousand Zobell's media throughout the study.

[c] Same as untreated systems, except that sediment was not added.

Appendix A-1. Description of acephate used in tests.

Acephate (Orthene). The technical material as supplied by the manufacturer was labeled "Chevron, ORTHO, ORTHENE, Analytical Standard, S X 985; Chevron Chemical Company, Ortho Division/San Francisco, CA 94119, Richmond, CA 94804" and "Orthene, t 169.5." Amount: ≈ 20 g.

EPA/FDA No. 481

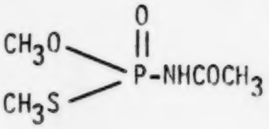
Lab Code: 73-31

Name: acephate; Orthene®; Ortho 12,420

Systematic Name: O,S-Dimethyl acetylphosphoramidothioate

Empirical Formula: $C_4H_{10}NO_3PS$ M.W. 183.17 State: Solid

Structure:



Use: Insecticide

Stability: Slightly hygroscopic, sensitive to heat, relatively stable to hydrolysis

Toxicity: Acute Oral LD_{50} (rats) female 866 mg/kg; male 945 mg/kg.

Mass spectral analysis was not run on this pesticide.

Appendix A-2. Description of aldicarb used in tests.

Aldicarb (Temik). The technical material was labeled "Aldicarb, Union Carbide, 5/2/78, Purity: 100%, Lot 4-CHA-32-2, amount: 1 gm."

The primary standard was obtained from the Pesticide Reference Standards Section of EPA and had the following characteristics:

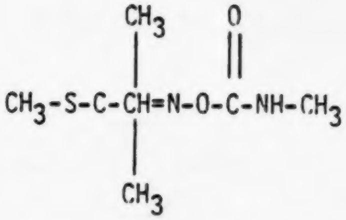
FDA No. 262

Name: aldicarb; Temik®, UC-21149

Systematic Name: 2-Methyl-2-(methylthio)propionaldehyde O-(methylcarbonyl) oxime

Empirical Formula: $C_7H_{14}N_2O_2S$ M.W. 190.26 State: Solid

Structure:



$$\mathrm{CH_3{-}S{-}C{-}\overset{\overset{CH_3}{|}}{\underset{\underset{CH_3}{|}}{CH}}{=}N{-}O{-}\overset{\overset{O}{\|}}{C}{-}NH{-}CH_3}$$

Stability: Decomposes on prolonged heating; oxidized by air to the sulfoxide and sulfone

Toxicity: Acute Oral LC_{50} (rats) 1 mg/kg

Purity: 99.8% ± 0.1%

Mass spectral analysis was not run on this pesticide.

Appendix A-3. Description of carbophenothion used in tests.

Carbophenothion (Trithion^R). The technical material was from a stock supply and was labeled "Carbophenothion, Chem Services, 12/77, 9/6/78, 6/6/79; Purity: 94.6%; amount: 10 gm."

The primary standard was obtained from the Pesticide Reference Standards Section of EPA and had the following characteristics:

FDA N. 147

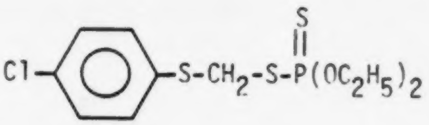
Lab Code: 70-66

Name: Carbophenothion; Trithion^R

Systematic Name: S-[(p-Chlorophenylthio)methyl] 0,0-diethyl phosphorodithioate

Empirical Formula: $C_{11}H_{16}ClO_2PS_3$ M.W. 342.87 State: Liquid

Structure:

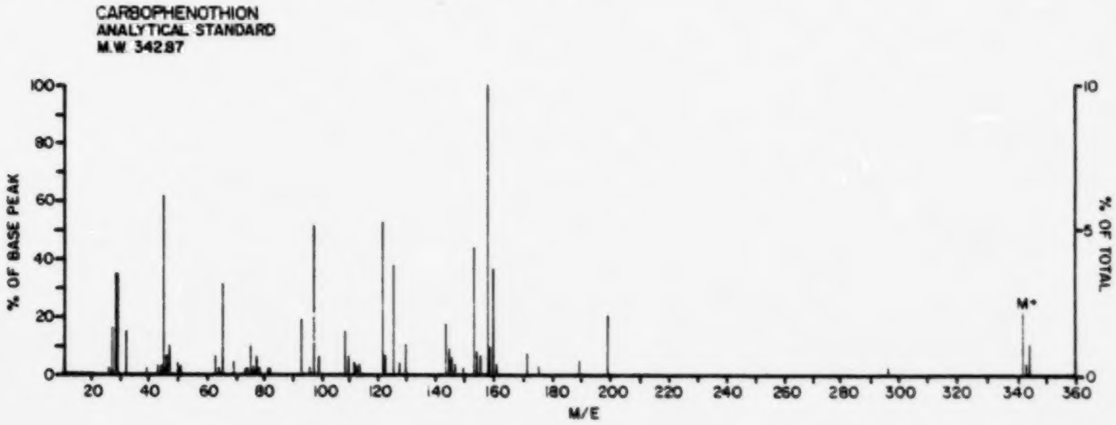


Stability: Relatively stable in water

Toxicity: Acute Oral LD_{50} (rats) 10 - 30 mg/kg. Absorbed through the skin.

Manufacturer's Assay: 95%

The mass spectra of the technical and analytical material were identical, with the molecular ion visible at M/E 343.



Appendix A-4. Description of DEF used in tests.

DEF. The technical material was from a stock supply and was labeled "DEF, Lot: 7710549, Chem Services, Date: 3/25/78, Purity: 95%, amount: 10 gm."

The primary standard was obtained from the Pesticide Reference Standards Section of EPA and had the following characteristics:

FDA No. 61
Lab Code: 68-33

Name: DEF®

Systematic Name: S,S,S-Tributyl phosphorotrithioate

Empirical Formula: $C_{12}H_{27}OPS_3$ M.W. 314.50 State: Liquid

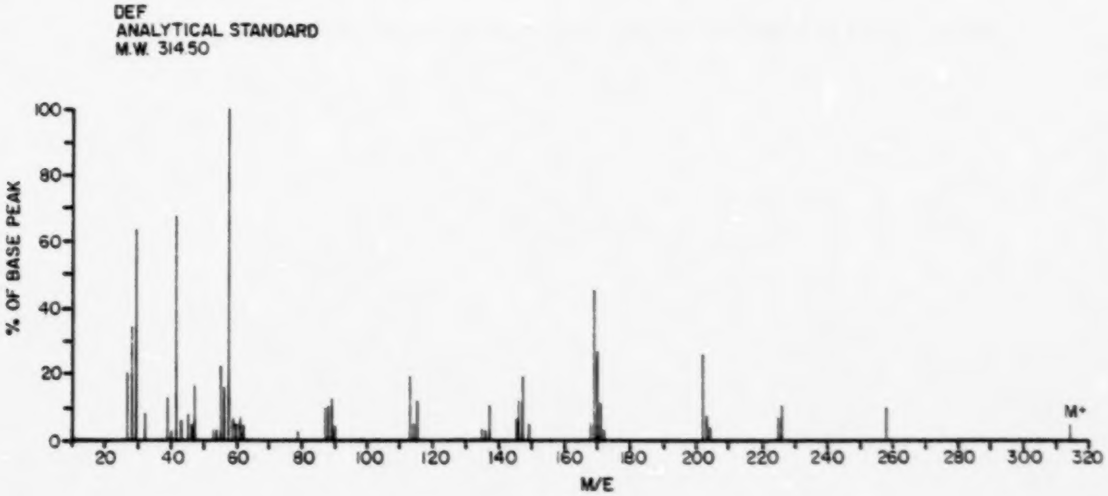
Structure: $(C_4H_9S)_3\ P{=}O$

Stability: Hydrolyzed by strong acid and alkali.

Toxicity: Acute Oral LD_{50} (rats) 325 mg/kg. Absorbed through the skin.

Manufacturer's Assay: Technical; approximately 94%

The mass spectra of the technical and analytical material were identical, with the molecular ion visible at M/E 314.



Appendix A-5. Description of Ethoprop used in tests.

Ethoprop (Mocap^R). The technical material was supplied by Bionomics Research Laboratory and was not labeled.

The primary standard was obtained from the Pesticide Reference Standards Section of EPA and had the following characteristics:

FDA No. 350

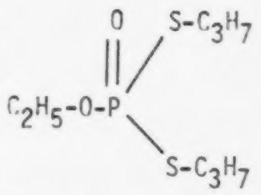
Lab Code: 68-106

Name: Mocap®; Prophos®

Systematic Name: O-Ethyl S,S,-dipropyl phosphorodithioate

Empirical Formula: $C_8H_{19}O_2PS_2$ M.W 242.34 State: Liquid

Structure:



Stability: Hydrolyzed by alkali

Toxicity: Acute Oral LD_{50} (rats) 30-56 mg/kg. Absorbed through the skin.

Manufacturer's Assay: 95%

Mass spectral analysis was not run on this pesticide.

Appendix A-6. Description of EPN used in tests.

EPN. The technical material was obtained from a stock supply and was labeled "EPN, Supplier: Chem Services, Date: 3/25/78, Purity: 97.2%, amount: 10 gm."

The primary standard was obtained from the Pesticide Reference Standards Section of EPA and had the following characteristics:

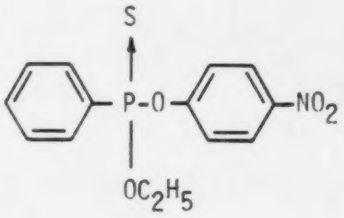
FDA No. 51

Name: EPN

Systematic Name: O-Ethyl O-p-nitrophenyl phenylphosphonothioate

Emphirical Formula: $C_{14}H_{14}NO_4PS$ M.W. 323.31 State: Solid

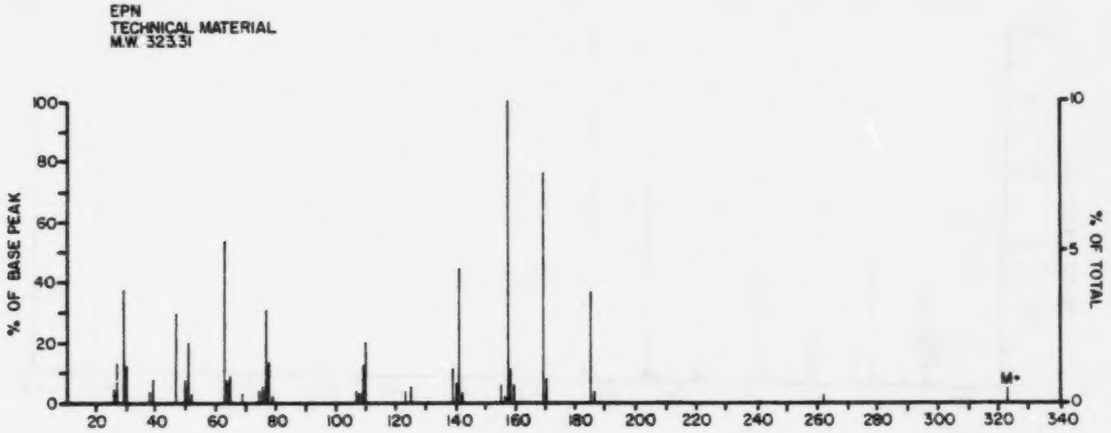
Structure:



Stability: Unstable in alkali

Toxicity: Acute Oral LD_{50} (rats) 9-45 mg/kg. Absorbed through the skin.

The mass spectra of the technical and analytical material were identical, with the molecular ion visible at M/E 323.



Appendix A-7. Description of methyl parathion used in tests.

Methyl Parathion. The technical material was obtained from a stock supply that was labeled "Methyl Parathion, MGF: Chem Services, Date: 12/9/77, Purity: 99%, amount: 10 gm."

The primary standard was obtained from the Pesticide Reference Standards Section of EPA and had the following characteristics:

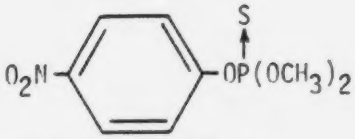
FDA No. 88

Name: Methyl Parathion

Systematic Name: 0,0-Dimethyl 0,p-nitrophenyl phosphorothioate

Emphirical Formula: $C_8H_{10}NO_5PS$ M.W. 263.21 State: Solid

Structure:

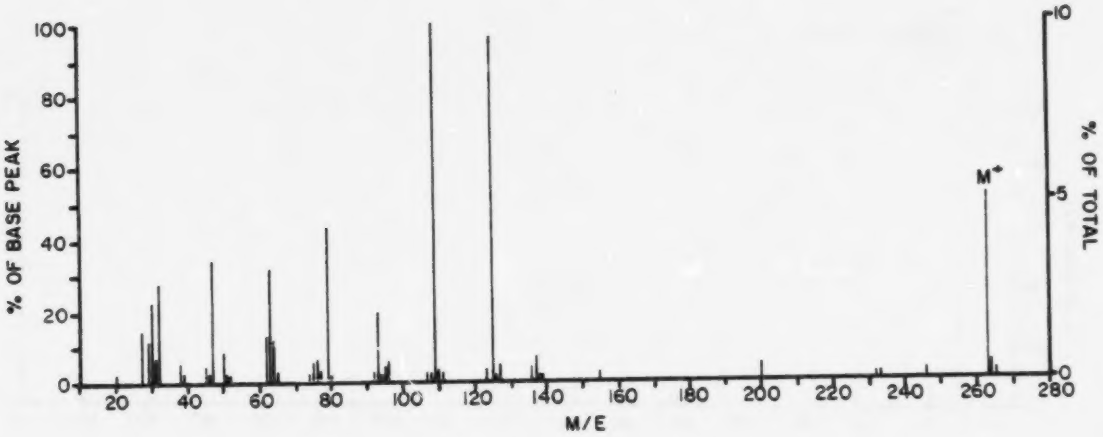


Stability: Hydrolized in alkali.

Toxicity: Acute Oral LD_{50} (rats) 9-25 mg/kg. Absorbed through the skin.

The mass spectra of the technical and analytical material were identical, with the molecular ion visible at M/E 263.

METHYL PARATHION
TECHNICAL MATERIAL
M.W. 263.21



Appendix A-8. Description of phorate used in tests.

Phorate (Thiomet^{R}). The technical material was from a stock supply and was labeled "Phorate (Thiomet), Lot: SPS-11548, American Cyanamid, Date: 12/77, Purity: 89.5%, amount: 5 gm."

The primary standard was obtained from the Pesticide Reference Standards Section of EPA and had the following characteristics:

FDA No. 160

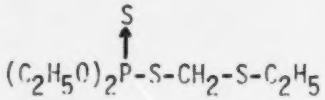
Lab Code: 70-14

Name: phorate; Thiomet^{R}

Systematic Name: O-O-Diethyl S-(ethylthio)methyl phosphorodithioate

Empirical Formula: $C_7H_{17}\ O_2PS_3$ M.W. 260.38 State: Liquid

Structure:

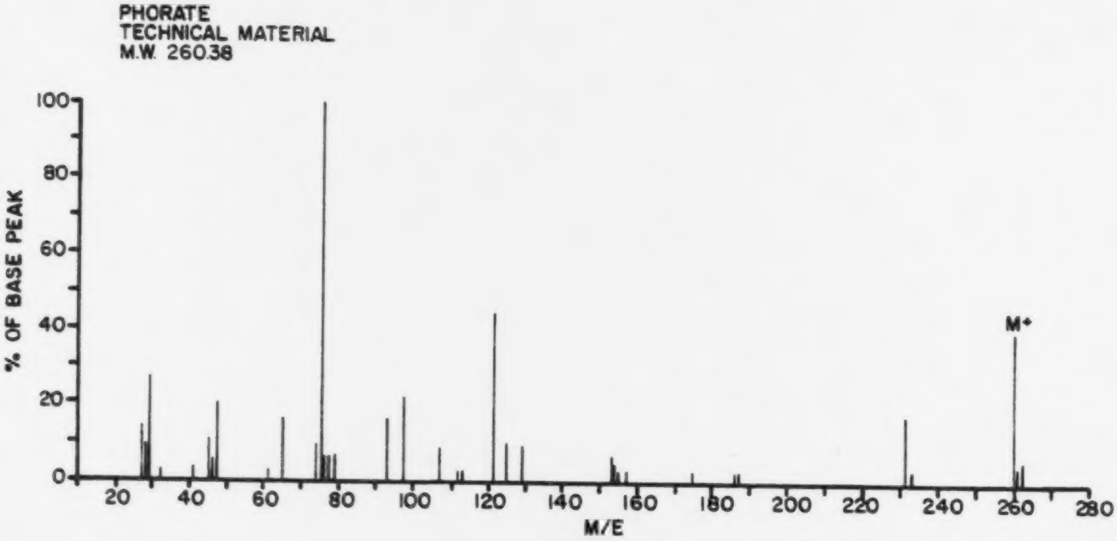


$$(C_2H_5O)_2\overset{\overset{S}{\uparrow}}{P}-S-CH_2-S-C_2H_5$$

Stability: Hydrolyzed by alkali

Toxicity: Acute Oral LD_{50} (rats) 1-2 mg/kg. Absorbed through the skin.

The mass spectra of the technical and analytical material were identical, with the molecular ion visible at M/E 260.



APPENDIX B

CHEMICAL METHODS FOR THE ANALYSES OF ACEPHATE (ORTHENE^R), ALDICARB (TEMIK^R), CARBOPHENOTHION (TRITHION^R), ETHOPROP (MOCAP^R), AND METHYL PARATHION IN SEAWATER AND TISSUE SAMPLES

By

EG&G International, Inc., Bionomics Operations
Bionomics Marine Research Laboratory
Route 6, Box 1002
Pensacola, Florida 32507

In Partial fulfillment of:
Contract Number 68-01-4974

U.S. Environmental Protection Agency
Ecological Effects Branch
Office of Pesticide Programs
Washington, D.C. 20460

The chemical methods described in this report were used for the quantification of acephate (Orthene®), aldicarb (Temik®), carbophenothion (Trithion®), ethoprop (Mocap®), and methyl parathion in seawater and tissue samples from toxicity tests conducted at Bionomics Marine Research Laboratory (BMRL) in Pensacola, Florida. This report should be referred to in conjunction with the following reports submitted to the U.S. Environmental Protection Agency: BP-79-2-16, BP-79-2-17, BP-79-2-18, BP-79-2-19, and BP-79-2-22.

All data related to these studies are stored at BMRL.

All chemicals used in preparing the seawater and tissue samples for chemical analysis were pesticide grade.

CHEMICAL METHODS

Water samples for acephate (Orthene)

Seawater samples (900-milliliters [mℓ]) were placed in amber glass bottles with Teflon®-lined caps. Each sample was preserved with 15 mℓ of chloroform and stored in the dark at ambient temperature until analyzed.

A 25-mℓ subsample was removed from the sample bottle and added to a 0.5-liter (ℓ) jar. One hundred grams (g) of anhydrous sodium sulfate was added to the jar with stirring to prevent caking. The sodium sulfate had been washed with ethyl acetate and oven dried at 275 degrees Celsius (°C) to eliminate interfering components. Then 100 mℓ of ethyl acetate was quickly added and blended with the sodium sulfate at high speed for two minutes with a top-drive blender.

The solids were allowed to settle and the extract was decanted through a glass funnel plugged with glass wool and containing approximately 50 of g sodium sulfate. The extraction and decanting were repeated twice with 50-mℓ portions of ethyl acetate. The sodium sulfate was then washed with a 25-mℓ portion of ethyl acetate and then the combined solvent fractions were collected in a round bottom flask and evaporated to less than 1 mℓ on a rotary evaporator with a 40-45°C water bath.

The sample was then transferred to a 15-mℓ graduated centrifuge tube with methyl-isobutyl-ketone and diluted to a known volume. Finally, the sample was transferred to a glass vial, capped with a Teflon-faced rubber septum and stored at 8°C until analyzed.

In order to verify the recovery of acephate from seawater, quality control standards were produced by fortifying seawater with known masses of acephate dissolved in acetone. The samples were prepared for analysis by the preceding methodology. Analytical standards of acephate in methyl-isobutyl-ketone were prepared by serial dilution of the acephate-in-acetone stock solution.

The samples were analyzed by using the following instrument conditions:

Instrument: Perkin-Elmer Model Sigma 2 gas chromatograph equipped with a rubidium bead nitrogen-phosphorus detector.

Column: 3-m x-2 mm ID Pyrex® packed with 10% carbowax 20 M on 80/100 mesh Supelcoport

Temperatures (°C): Injector: 225
Oven: 200
Detector: 250

Gas flows: Carrier: nitrogen, 20 mℓ/minutes (min)
Reactant: hydrogen, 5 mℓ/min
Support: air, 100 mℓ/min

Retention time: 2.2 min

Chart speed: 1 centimeter (cm)/min

Amplifier range: 1 millivolt (mv)

Attenuation: 1

Bead adjust: 400

Response: Approximately half-chart deflection with 60 ng acephate

The results of the recovery of acephate from seawater are summarized in the following table.

Sample and replicate	Acephate mass added (mg)	Acephate mass recovered (mg)	Percentage recovery
Blank A	0.0	<0.01	N/A
B	0.0	<0.01	N/A
C	0.0	<0.007	N/A
D	0.0	<0.008	N/A
E	0.0	<0.005	N/A
Low range A	0.05	0.018	36
B	0.05	0.030	60
C	0.05	0.040	80
D	0.05	0.025	50
E	0.05	<0.007	---
Mid range A	0.50	0.22	44
B	0.50	0.17	34
C	0.50	0.19	38
D	0.50	0.11	22
E	0.50	0.32	64
High range A	5.0	2.4	48
B	5.0	2.4	48
C	5.0	3.2	64
D	5.0	4.0	80
E	5.0	3.1	62

Mean percentage recovery ± standard deviation: 52±17.

Low range E was not included in the calculation of the mean.

The analytical results of the samples were calculated by comparing the peak height at retention time 2.2 minutes with the peak heights of external acephate standards. A calibration curve was calculated using the parabolic curve fit with the sum of the squares of the errors minimized (Adams, 1976) for the peak heights (mm) versus mass (ng) of acephate. The results were corrected for a 52% recovery. The minimum detectable concentration of acephate in seawater was 0.4 mg/ℓ based on a 25-mℓ water sample concentrated to 5.0 mℓ.

Water samples for aldicarb (Temik)

Seawater samples (900-mℓ) were placed in amber glass bottles with Teflon-lined caps. Each sample was preserved with 15 mℓ of chloroform and stored in the dark at ambient temperature until analyzed.

Samples were extracted twice in a 1-ℓ separatory funnel with 50 mℓ portions of dichloromethane. The organic phase was passed through glass wool to break up any emulsions. The pooled organic phase plus 2 mℓ toluene were evaporated almost to dryness on a rotary evaporator in a water bath at 40-50°C.

The samples were then quantitatively transferred to a 15-mℓ graduated centrifuge tube with toluene and diluted to a known volume. The sample was then transferred to a clear glass vial and capped with a Teflon-sided septum. The sample was stored in a refrigerator at 8°C prior to analysis.

In order to verify the recovery of aldicarb from seawater, quality control standards were produced by fortifying seawater with known masses of aldicarb dissolved in acetone. The samples were then prepared for analysis by the preceding method. Analytical standards of aldicarb in toluene were produced by serial dilution of stock aldicarb solutions in toluene. A calibration curve was calculated using the parabolic curve fit with the sum of the squares of the errors minimized (Adams, 1976) for the peak heights (mm) versus mass (ng) of aldicarb.

The samples were analyzed by using the following instrument conditions:

Instrument: Perkin-Elmer Model Sigma 2 gas chromatograph equipped with a rubidium bead nitrogen/phosphorus detector

Column: 3-m x-2 mm ID Pyrex packed with 10% carbowax 20 M on 80/100 mesh Supelcoport

Temperatures (°C): Injector: 225
Oven: 140
Detector: 250

Gas flows: Carrier: nitrogen, 50 mℓ/min
Reactant: hydrogen, 5 mℓ/min
Support: air, 100 mℓ/min

Retention time: 1.1 min

Chart speed: 1 cm/min

Amplifier range: 1 mv

Attenuation: 1

Bead adjust: 370

Response: Approximately half-chart deflection with 1.9 ng aldicarb

The results of the recovery of aldicarb from seawater are summarized in the following table.

Sample and replicate	Aldicarb mass added (µg)	Aldicarb mass recovered (µg)	Percentage recovery
Blank A	0.0	<.02	N/A
B	0.0	<.02	N/A
C	0.0	<.02	N/A
D	0.0	<.02	N/A
E	0.0	<.02	N/A
Low range A	3.2	2.6	81
B	3.2	2.4	75
C	3.2	2.0	62
D	3.2	2.0	62
E	3.2	1.7	53
Mid range A	32	40	120
B	32	25	78
C	32	18	56
D	32	20	62
E	32	23	72
High range A	320	210	66
B	320	210	66
C	320	270	84
D	320	270	84
E	320	240	75

Mean percentage recovery ± standard deviation: 70±10.

Mid range A was not included in the calculation of the mean.

The analytical results of the samples were calculated by comparison of the peak height with the retention time of 1.1 minutes with the peak heights of aldicarb external standards. The results were corrected for a 70% recovery. The minimum detectable concentration of aldicarb in seawater was 0.2 μg/ℓ based on a 900-mℓ seawater sample being concentrated to 5.0 mℓ.

Water samples for ethoprop (Mocap), carbophenothion (Trithion), and methyl parathion

Seawater samples (900-mℓ) were placed in amber glass bottles with Teflon-lined caps. Each sample was preserved with 15 mℓ of chloroform and stored in the dark at ambient temperature.

Seawater samples were extracted twice with 50-mℓ aliquots of dichloromethane. The pooled organic phase was passed through a 20-mm ID chromatography column containing 50 mm of anhydrous sodium sulfate which had been dried overnight at 275°C. The sodium sulfate was then washed with an additional 30 mℓ of dichloromethane.

The organic phase was collected in a 500-mℓ Kuderna-Danish evaporator equipped with a 10-mℓ collector. A porcelain boiling chip was added to the evaporator which was then fitted with a three-ball Snyder column. The solvent was concentrated to approximately 3 mℓ on a steam bath. The evaporator was cooled and then rinsed with 2 mℓ hexane.

The collector tube was fitted with a Micro-Snyder column, placed into an 80°C Kontes tube heater and the solvent was then concentrated to less than 0.5 mℓ.

The sample was quantitatively transferred to a 15 mℓ-graduated centrifuge tube and diluted to a known volume with hexane. The sample was transferred to a clear glass vial capped with a Teflon®-faced rubber septum and stored at 8°C until analyzed.

The samples were analyzed by using the following instrument conditions:

Instrument: Perkin-Elmer Model Sigma 2 gas chromatograph equipped with a rubidium bead nitrogen/phosphorus detector

Column: 2-m x-2 mm Pyrex packed with 3% OV-1 on 100/120 mesh Supelcoport

Temperatures (°C): Injector: 225
Detector: 250
Oven temperature program:

	Initial	Final	Post
Oven temp. (°C)	145	200	205
Time (min)	2.0	3.0	1.0
Rate (°C/min)	40	10	

Gas flows: Carrier: nitrogen, 60 mℓ/min
Reactant: hydrogen, 3 mℓ/min
Support: air, 100 mℓ/min

Amplifier range: 1 mv

Detector setting: 520

	Ethoprop	Methyl parathion	Carbophenothion
Retention time (min):	2.6	4.0	7.2
Attenuation:	8	128	4
Response (half-chart deflection):	0.44 ng	4.6 ng	0.35 ng

In order to quantitate the recovery of ethoprop, carbophenothion, and methyl parathion from seawater, quality control samples were produced by fortifying seawater with known masses of each pesticide dissolved in acetone. The samples were prepared for analysis by the preceding method.

Analytical standards were prepared by dilution of the stock solutions with hexane. A calibration curve was produced by calculating the parabolic curve fit of the sum of the squares of the errors minimized for the peak heights (mm) versus mass (ng) of the pesticides (Adams, 1976).

The analytical results of the samples were calculated by comparison of the peak heights found at 2.6, 4.0, and 7.2 minutes for ethoprop, methyl parathion, and carbophenothion, respectively.

Sample and replicate	Ethoprop mass added (µg)	Ethoprop mass recovered (µg)	Percentage recovery
Blank A	0.0	<0.01	N/A
B	0.0	<0.01	N/A
C	0.0	<0.01	N/A
D	0.0	<0.01	N/A
E	0.0	<0.01	N/A
Low range A	0.073	0.059	81
B	0.073	0.047	64
C	0.073	0.038	52
D	0.073	0.044	60
E	0.073	0.043	60
Mid range A	0.73	0.50	68
B	0.73	0.56	77
C	0.73	0.52	71
D	0.73	0.64	88
E	0.73	0.59	81

(continued)

continued

Sample and replicate	Ethoprop mass added (µg)	Ethoprop mass recovered (µg)	Percentage recovery
High range A	7.3	5.0	68
B	7.3	6.3	86
C	7.3	6.0	82
D	7.3	4.3	55
E	7.3	5.0	68

Mean percentage recovery ± standard deviation: 71±11.

Sample and replicate	Methyl parathion mass added (µg)	Methyl parathion mass recovered (µg)	Percentage recovery
Blank A	0.0	<0.1	N/A
B	0.0	<0.1	N/A
C	0.0	<0.1	N/A
D	0.0	<0.1	N/A
E	0.0	<0.1	N/A
Low range A	1.5	1.6	140
B	1.5	Lost	Lost
C	1.5	1.3	87
D	1.5	1.2	80
E	1.5	1.1	73
Mid range A	15	11	73
B	15	12	80
C	15	10	67

(continued)

continued

Sample and replicate	Methyl parathion mass added (µg)	Methyl parathion mass recovered (µg)	Percentage recovery
Mid range D	15	9.3	62
E	15	15	100
High range A	76	73	96
B	76	73	96
C	76	77	100
D	76	50	66
E	76	67	88

Mean percentage recovery ± standard deviation: 86±20.

Sample and replicate	Carbophenothion mass added (µg)	Carbophenothion mass recovered (µg)	Percentage recovery
Blank A	0.0	<0.03	N/A
B	0.0	<0.03	N/A
C	0.0	<0.03	N/A
D	0.0	<0.03	N/A
E	0.0	<0.03	N/A
Low range A	0.078	0.087	110
B	0.078	0.063	86
C	0.078	0.073	94
D	0.078	0.073	94
E	0.078	0.080	100

(continued)

continued

Sample and replicate	Carbophenothion mass added (µg)	Carbophenothion mass recovered (µg)	Percentage recovery
Mid range A	0.78	0.93	120
B	0.78	0.93	120
C	0.78	0.80	100
D	0.78	0.93	120
E	0.78	0.93	120
High range A	7.8	8.7	110
B	7.8	8.7	110
C	7.8	9.0	120
D	7.8	6.7	85
E	7.8	8.7	110

Mean percentage recovery ± standard deviation: 110±10.

The analytical results of ethoprop and methyl parathion were corrected for a 71% and 86% recovery, respectively. Carbophenothion results were not corrected for percentage recovery.

Tissue samples for acephate (Orthene) and aldicarb (Temik)

Acephate (Orthene) residues in marine tissues were semiquantitated by thin-layer chromatography (TLC) according to procedures of the U.S. EPA (1974) for organophosphorus pesticides except as mentioned. Methylcyclohexane was not used as the developing solvent because it was of insufficient polarity to cause acephate to migrate from the origin. Additionally, because a high polarity migrating solvent (acetone) was used, dimethylformamide was not used as the immobile solvent.

Tissue samples were weighed and extracted with two portions of acetone solvent using a Brinkman Polytron® homogenizer with a size PT-10 rotor at 27,000 rpm. The acetone extracts were combined, centrifuged to remove solids, transferred to a 50-mℓ volumetric flask, and diluted to a 50-mℓ volume with acetone.

Tissue extracts were spotted on Silica Gel (mean particle size of 250-μm) 60 glass plates. Acephate standards or tissue extracts (50-μℓ) were spotted 1.25 cm from the bottom of the plate and developed with 50% acetone-in-hexane. The solvent front traveled 150 mm to a line near the top of the plate. Acephate was developed by spraying with p-nitrobenzyl pyridine, heating the plate at 110°C for 10 minutes, and overspraying the plate with tetraethylenepentamine. The R_f (reference front) value of acephate standards and acephate spotted with tissue extract was 0.14.

Cleanup of the extract prior to TLC was not necessary because the fats and oils present migrated further up the plate with the migrating solvent used and the compound detection chemicals are known to react only with thio- and non thio-organophosphates. Acephate was detectable at 0.1 μg as a pale blue spot on a pale yellow background. The blue color intensity ranged from pale blue with 0.1 μg to dark blue with 1.0 μg of acephate. Spots containing in excess of 1.0 μg of acephate were of intense and equal dark blue color, therefore, all tissue sample extract volumes were spotted to contain between 0.1 to 1.0 μg of acephate.

Acephate spots present in chromatograms of tissue extract were of larger diameter than the corresponding weight of acephate standard, probably because of the larger volume of tissue extract spotted or the presence of extraneous biogenic materials which affected the migration of acephate. Acephate did appear as symmetrical circular spots in the chromatogram of tissue extracts and the spots were well resolved from other spots present on the chromatogram.

Quantitation was accomplished by comparing acephate standards and unknowns to estimate the amount present in the unknown sample based on an equivalent intensity of blue color. Next, the areas of the acephate standard and sample spots were measured and the ratio of sample to standard spot area was multiplied by the weight of acephate (derived by color intensity) to give the total weight present in the sample. For example, assuming that an acephate sample spot color intensity corresponded to 0.5 μg of acephate standard and that the sample and standard spots were 50 mm^2 and 10 mm^2, respectively. The weight of acephate present in the sample would be calculated as follows:

$$0.5\ \mu\text{g X } \frac{50\ \text{mm}^2}{10\ \text{mm}^2} = 2.5\ \mu\text{g total}$$

Acephate added to three pinfish tissues at 5.0 ppm and analyzed by TLC as described above was quantitatively recovered. The minimum detectable concentration (MDC) of acephate was 5.0 μg/g (wet weight) of tissue extracted.

Aldicarb (Temik) residues in marine tissue were semiquantitated by thin-layer chromatography. Tissue samples and aldicarb standards were oxidized to the sulfone and extracted according to the procedures outlined in Pesticide Analytical Manual (FDA, 1970), Method I, Step 2, Extraction-Oxidation. A portion of the extract was spotted on 250-μm Silica Gel 60 glass plates using 2:1 hexane-acetone migrating solvent system. The solvent front was 150 mm and the R_f for aldicarb sulfone was 0.29. Spots were developed by spraying with 0.2 per cent ninhydrin and 2 per cent pyridine-in-acetone, according to the procedures in Handbook of Chromatography (1972). Resulting spots were purple in color.

Aldicarb was not detected in any of the tissue samples analyzed by thin-layer chromatography as described above. Quantitative recovery of aldicarb was demonstrated by fortifying three pinfish tissue samples with 1.0 μg aldicarb/g tissue and analyzing the extract as described. Although the acephate sulfone spots present in fortified samples were larger in diameter than the equivalent weight of aldicarb sulfone standard, no streaking of the spot occurred in the chromatogram of fortified samples and the spot of aldicarb sulfone was well-resolved from the other spots present.

The larger spots due to aldicarb sulfone in sample chromatograms, compared to standards, were probably caused by the larger volume of tissue extract spotted or to the effect of extraneous biogenic material on the migration of aldicarb sulfone in the sample chromatogram. The minimum detectable weight of aldicarb sulfone was 1.0 μg/g (wet weight) of tissue analyzed.

Tissue samples for ethoprop (Mocap), carbophenothion (Trithion), and methyl parathion

Frozen tissue samples were prepared for gas chromatography by extracting the sample three times with 20-mℓ portions of dichloromethane for one minute using a Polytron PT20 homogenizer. The sample was centrifuged between extractions and the extract was decanted into a 100-mℓ Griffin beaker. The extract was concentrated over a steam bath to 5 mℓ.

The concentrate was transferred to a 1-x 30-cm Pyrex chromatographic column containing 10 cm of activated (130°C) Florisil (60/100 mesh) with a 1-cm surface layer of anhydrous sodium sulfate. The column was pre-rinsed with 50 mℓ of 30% ethyl ether in petroleum ether and then with 35 mℓ of petroleum ether before sample application.

The column was eluted with a 75-mℓ volume of 1:1 ethyl ether: petroleum ether. The eluant was collected in a 500-mℓ Kuderna-Danish evaporator equipped with a porcelain boiling chip and a 10-mℓ graduated collector. The concentrator was fitted with a three-ball Snyder column and placed on a steam bath until the eluant concentrated to approximately 5 mℓ. The sample was diluted to a known volume with hexane, transferred to a glass vial capped with a Teflon-faced rubber septum, and stored at 8°C until analysis.

Gas chromatographic analyses were performed by using the following instrument conditions:

Instrument: Perkin-Elmer Model Sigma 2 gas chromatograph equipped with a rubidium bead nitrogen-phosphorus detector.

Column: 2-m x 2-mm Pyrex packed with 3% OV-1 on 100/120 mesh Supelcoport

Temperatures (°C): Injector: 225
Detector: 250
Oven temperature program:

	Initial	Final
Oven temp. (°C)	180	250
Time (min)	1.0	1.1
Rate (°C/min)	40	

Gas flows: Carrier: nitrogen, 60 mℓ/min
Reactant: hydrogen, 5 mℓ/min
Support: air, 100 mℓ/min

Chart speed: 1 cm/min

Amplifier range: 10

Bead setting: 370

	Ethoprop	Methyl parathion	Carbophenothion
Retention time (min)	0.9	2.0	3.5
Attenuation:	4	16	16
Response (half-chart deflection):	0.8 ng	4.5 ng	7.2 ng

Calibration curves were produced by plotting peak heights (mm) verses mass (ng) of standard injected. Analytical standards were prepared by dilution of analytical pesticide standards with hexane to yield working standards of the required concentrations.

Unexposed tissue (∿1 g) was fortified with pesticide standards-in-acetone and analyzed by the preceding method. The analytical results of all samples were corrected for the mean percentage recoveries shown in the following tables.

Sample and replicate	Ethoprop mass added (μg)	Ethoprop mass recovered (μg)	Percentage recovery
Blank A	0.0	<0.03	N/A
B	0.0	<0.03	N/A
C	0.0	<0.03	N/A
D	0.0	<0.03	N/A
E	0.0	<0.03	N/A
Low range A	0.14	0.12	86
B	0.14	0.12	86
C	0.14	0.17	120
D	0.14	0.095	68
E	0.14	0.13	93
Mid range A	1.4	1.1	78
B	1.4	1.1	78
C	1.4	1.0	71
D	1.4	0.77	55
E	1.4	0.85	61
High range A	14	13	93
B	14	4	28
C	14	12	86
D	14	11	78
E	14	13	93

Mean percentage recovery ± standard deviation: 82±16.

High range B was not used to calculate mean percentage recovery.

Sample and replicate	Methyl parathion mass added (μg)	Methyl parathion mass recovered (μg)	Percentage recovery
Blank A	0.0	<0.02	N/A
B	0.0	<0.02	N/A
C	0.0	<0.02	N/A
D	0.0	<0.02	N/A
E	0.0	<0.02	N/A
Low range A	0.15	0.30	200
B	0.15	0.14	93
C	0.15	0.13	87
D	0.15	0.16	110
E	0.15	0.14	93
Mid range A	1.5	1.0	67
B	1.5	0.97	65
C	1.5	0.85	57
D	1.5	0.92	61
E	1.5	0.93	62
High range A	15	13	87
B	15	<1	---
C	15	12	80
D	15	9.7	65
E	15	11	73

Mean percentage recovery ± standard deviation: 77±16.

Low range A and High range B were not used to calculate mean percentage recovery.

Sample and replicate	Carbophenothion mass added (μg)	Carbophenothion mass recovered (μg)	Percentage recovery
Blank A	0.0	<0.02	N/A
B	0.0	<0.02	N/A
C	0.0	<0.02	N/A
D	0.0	<0.02	N/A
E	0.0	<0.02	N/A
Low range A	0.12	0.053	44
B	0.12	0.087	72
C	0.12	0.023	19
D	0.12	0.060	50
E	0.12	0.067	56
Mid range A	1.2	0.52	43
B	1.2	0.50	42
C	1.2	0.62	52
D	1.2	0.52	43
E	1.2	0.07	5.8
High range A	12	8.7	72
B	12	<2	---
C	12	7.0	58
D	12	4.7	39
E	12	6.3	52

Mean percentage recovery ± standard deviation: 52±11.

Low range C, Mid range E, and High range B were not used to calculate the mean percentage recovery.

The detection limits of ethoprop, methyl parathion, and carbophenothion in tissue were 0.03, 0.02, and 0.02 μg, respectively, based on a sample mass of 1 gm.

REFERENCES

Adams. 1976. Application programs. International Software Clearinghouse. Vol 1: 15-18

U.S. Environmental Protection Agency. 1974. Manual of analytical methods for the analysis of pesticide residues in human and environmental samples. Environmental Toxicology Division, Research Triangle Park, N.C. Section 123: 1-14.

U.S. Food and Drug Administration. 1970. Pesticide Analytical Manual. Method I and Method A, Temik. Vol II: Section 120-269

Zweig, Gunter, editor. 1972. Handbook of Chromatography. Chemical Rubber Co, Cleveland, OH. Vol II: 117

PREPARED BY: Peter J. Shuba, Ph.D.

Peter J. Shuba

Senior Scientist

REVIEWED BY: Peter J. Shuba, Ph.D.

Peter J. Shuba

Senior Scientist

APPROVED BY: Rod Parrish

Rod Parrish/PS

Director

Appendix C-1. Acute (96-h) toxicity of eight pesticides to the marine diatom (*Skeletonema costatum*) in static tests. Effect measured was specific conductance; triplicate samples were measured for each concentration and control.

Insecticide (96-hour EC50, µg/ℓ)		Concentration (µg/ℓ)	96-h Absorbance (@ 625 nm)	Mean Absorbance
Acephate (>50,000)	Trial 1:	Control	0.089,0.072,0.085	0.082
		100	0.068,0.061,0.086	0.072
		1,000	0.086,0.091,0.087	0.088
		5,000	0.087,0.090,0.071	0.083
		10,000	0.081,0.088,0.084	0.084
	Trial 2:	Control	0.083,0.073,0.100	0.085
		100	0.092,0.087,0.092	0.090
		1,000	0.082,0.096,0.084	0.087
		5,000	0.088,0.087,0.090	0.088
		10,000	0.088,0.078,0.102	0.089
	Trial 3:	Control	0.080,0.077,0.073	0.077
		10,000	0.098,0.074,0.087	0.086
		25,000	0.087,0.071,0.101	0.086
		50,000	0.083,0.092,0.082	0.086
Aldicarb (>50,000)	Trial 1:	Control	0.060,0.031,0.049	0.046
		100	0.041,0.052,0.034	0.042
		1,000	0.042,0.053,0.046	0.047
		5,000	0.040,0.050,0.037	0.042
		10,000	0.030,0.057,0.041	0.043
	Trial 2:	Control	0.088,0.092,0.088	0.089
		100	0.065,0.068,0.080	0.071
		1,000	0.087,0.080,0.072	0.080
		5,000	0.080,0.105,0.079	0.088
		10,000	0.081,0.090,0.087	0.086
	Trial 3:	Control	0.094,0.076,0.078	0.083
		10,000	0.080,0.876,0.068	0.075
		25,000	0.080,0.074,0.075	0.076
		50,000	0.077,0.075,0.072	0.075
Carbo-phenothion (109)	Trial 1:	Control	0.123,0.121,0.103	0.116
		1	0.097,0.108,0.118	0.108
		10	0.116,0.097,0.099	0.104
		100	0.109,0.082,0.088	0.093
		1,000	0.012,0.012,0.016	0.013
		5,000	0.018,0.018,0.015	0.017
		10,000	0.000,0.015,0.008	0.008

Carbophenothion (continued)

	Trial 2:	Control	0.131,0.136,0.140	0.136
		100	0.084,0.072,0.058	0.071
		125	0.066,0.022,0.022	0.037
		150	0.041,0.018,0.018	0.026
		175	0.007,0.007,0.008	0.007
		200	0.004,0.007,0.007	0.006
	Trial 3:	Control	0.084,0.110,0.108	0.101
		100	0.068,0.075,0.071	0.071
		200	0.021,0.016,0.020	0.019
		400	0.014,0.014,0.017	0.015
		600	0.011,0.019,0.015	0.015
		800	0.010,0.009,0.013	0.011
	Trial 4:	Control	0.099,0.098,0.091	0.096
		100	0.065,0.068,0.058	0.064
		200	0.019,0.019,0.018	0.019
		400	0.018,0.018,0.016	0.017
		600	0.013,0.012,0.015	0.013
		800	0.017,0.017,0.011	0.015
DEF (366)	Trial 1:	Control	0.110	0.110
		100	0.072,0.074,0.089	0.078
		200	0.059,0.076,0.050	0.062
		500	0.017,0.006,0.014	0.012
		700	0.014,0.020,0.015	0.016
		900	0.010,0.016,0.016	0.014
	Trial 2:	Control	0.123,0.118,0.095	0.112
		100	0.108,0.094,0.108	0.103
		200	0.115,0.106,0.098	0.106
		300	0.084,0.087,0.064	0.078
		400	0.070,0.074,0.057	0.067
		500	0.044,0.010,0.016	0.023
	Trial 3:	Control	0.093,0.099,0.099	0.097
		100	0.086,0.090,0.059	0.088
		200	0.077,0.074,0.064	0.072
		300	0.046,0.048,0.055	0.050
		400	0.047,0.038,0.033	0.039
		500	0.038,0.031,0.022	0.030
	Trial 4:	Control	0.142,0.138,0.121	0.134
		100	0.114,0.112,0.128	0.118
		200	0.108,0.104,0.089	0.100

DEF (continued)

		300	0.098,0.070,0.077	0.082
		400	0.055,0.066,0.024	0.048
		500	0.003,0.006,0.007	0.005
EPN (340)	Trial 1:	Control	0.106,0.120,0.098	0.108
		100	0.123,0.095,0.122	0.113
		200	0.094,0.078,0.096	0.089
		300	0.099,0.111,0.099	0.100
		400	0.090,0.063,0.074	0.076
		500	0.020,0.034,0.038	0.031
	Trial 2:	Control	0.151,0.170,0.151	0.157
		200	0.123,0.136,0.110	0.123
		300	0.088,0.087,0.096	0.090
		400	0.027,0.009,0.021	0.019
		450	0.016,0.015,0.018	0.016
		500	0.014,0.016,0.014	0.015
	Trial 3:	Control	0.143,0.146,0.163	0.151
		200	0.141,0.130,0.144	0.138
		300	0.130,0.120,0.133	0.129
		400	0.103	0.103
		450	0.028,0.022,0.024	0.025
		500	0.026,0.024,0.025	0.025
Ethoprop (8,400)	Trial 1:	Control	0.122,0.107,0.094	0.108
		1	0.117,0.103,0.103	0.108
		10	0.102,0.098,0.109	0.103
		100	0.113,0.099,0.110	0.107
		1,000	0.092,0.107,0.103	0.101
		5,200	0.057,0.055,0.051	0.054
	Trial 2:	Control	0.080,0.082,0.085	0.082
		1,040	0.082,0.099,0.082	0.088
		2,600	0.073,0.083,0.072	0.076
		5,200	0.050,0.061,0.041	0.051
		7,800	0.053,0.046,0.052	0.050
		10,400	0.036,0.034,0.046	0.039
	Trial 3:	Control	0.089,0.090,0.084	0.088
		1,040	0.090,0.076,0.090	0.083
		2,600	0.080,0.070,0.075	0.075
		5,200	0.078,0.062,0.055	0.065
		7,800	0.053,0.048,0.056	0.052
		10,400	0.011,0.012,0.014	0.012
Methyl parathion (5,300)	Trial 1:	Control	0.138,0.135,0.130	0.134
		1,000	0.143,0.145,0.123	0.137
		2,000	0.115,0.109,0.110	0.111
		3,000	0.087,0.081,0.080	0.083
		4,000	0.077,0.068,0.066	0.070
		5,000	0.057,0.058,0.052	0.056

Methyl Parathion (continued)

	Trial 2:	Control	0.143,0.138,0.128	0.136
		2,000	0.137,0.124,0.116	0.126
		3,000	0.127,0.140,0.084	0.117
		4,000	0.099,0.087,0.104	0.097
		5,000	0.071,0.066,0.065	0.067
		6,000	0.036,0.031,0.032	0.033
	Trial 3:	Control	0.146,0.143,0.149	0.146
		2,000	0.152,0.173,0.183	0.169
		3,000	0.130,0.146,0.168	0.148
		4,000	0.128,0.128,0.136	0.131
		5,000	0.098,0.116,0.110	0.108
		6,000	0.068,0.063,0.076	0.069
Phorate (1,300)	Trial 1:	Control	0.104,0.112,0.094	0.103
		1,000	0.063,0.054,0.074	0.064
		2,000	0.086,0.034,0.020	0.027
		3,000	0.020,0.024,0.017	0.020
		4,000	0.020,0.018,0.018	0.019
		5,000	0.018,0.016,0.014	0.016
	Trial 2:	Control	0.146,0.147,0.152	0.148
		1,000	0.091,0.078,0.091	0.087
		2,000	0.051,0.041,0.046	0.046
		3,000	0.011,0.010,0.015	0.012
		4,000	0.010,0.009,0.012	0.010
	Trial 3:	Control	0.140,0.156,0.136	0.144
		500	0.131,0.116,0.120	0.122
		1,000	0.109,0.108,0.107	0.108
		2,000	0.034,0.021,0.026	0.027

Appendix C-2a. Number of normal eastern oyster (Crassostrea virginica) embryos per milliliter counted following 48 h of exposure to acephate in static, unaerated seawater. Salinity was 20 °/oo and temperature, 25±1°C. If all embryos in the initial inoculum had developed, the expected count would have been 818 embryos per milliliter.

Nominal concentration (µg/ℓ)	Number of normal embryos				
	Rep[a] A	Rep B	Rep C	Mean	SD[b]
Control	638	542	604	594	±48
56,000	583	526	629	579	±51
100,000	509	523	441	491	±43
320,000	125	211	158	164	±43
560,000	0	0	0	0	0
1,000,000	0	0	0	0	0

[a] Replicate.
[b] Standard deviation.

Appendix C-2b. Toxicity of acephate to embryos of eastern oysters (Crassostrea virginica) exposed for 48 h in static, unaerated seawater. Criterion of effect was reduction of the number of normal embryos in test concentrations as compared to the number of normal control embryos. Salinity was 20 °/oo and temperature 25±1°C.

Nominal concentration (μg/ℓ)	Percentage reduction[a]
Control	--
56,000	2
100,000	17
320,000	73
560,000	100
1,000,000	100

$$^{a}\text{Percentage reduction} = \frac{\text{Number of normal 48-h control embryos minus the number of normal 48-h embryos in each test concentration}}{\text{Number of normal 48-h control embryos}} \times 100$$

Appendix C-3. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to acephate in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 ^{o}/oo; temperature was 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
1,000	0	0	0	0
3,200	0	0	0	0
10,000	0	0	0	0

[a] Hours.

Appendix C-4. Test concentrations and mortality of sheepshead minnows (Cyprinodon variegatus) exposed to acephate in static acute toxicity tests using 20 fish per concentration. Natural seawater was adjusted to 20 ‰; temperature was 25±1°C.

Nominal concentration (μg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
320,000	0	0	0	0
560,000	0	0	0	0
1,000,000	0	0	0	10
1,800,000	0	0	0	10
3,200,000	0	0	0	10

[a] Hours

Appendix C-5. Test concentrations and mortality of spot (Leiostomus xanthurus) exposed to acephate in static acute toxicity tests using 30 animals per concentration. Natural seawater was adjusted to 20 ‰; temperature was 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
100,000	0	10	17	23

[a] Hours.

Appendix C-6. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to acephate in flowing, natural seawater of 29±1 $^{o}/oo$ salinity and 22±1°C. Twenty animals were tested in each treatment.

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
13,000	ND	0	0	15	30
22,000	3,500	0	0	15	35
36,000	7,400	0	0	10	50
60,000	15,000	0	15	40	65
100,000	32,000	0	5	30	75

[a] Hours.
[b] Nondetectable (<400 μg/ℓ).

Appendix C-7. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to acephate in flowing, natural seawater of 28-30 $^{0}/oo$ salinity and $22 \pm 1^{o}C$. Twenty animals were tested in each treatment.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
13,000	ND	0	0	0	0
22,000	3,500	0	0	15	70
36,000	7,400	0	15	50	55
60,000	15,000	0	40	100	100
100,000	32,000	15	75	100	100

---

[a] Hours.

[b] Nondetectable (<400 µg/ℓ).

Appendix C-8. Ranges of dissolved oxygen and pH measured in seawater from acute static and flow-through toxicity tests.

Insecticide	Species	Test	Dissolved oxygen (µg/ℓ, % Saturation)		pH
Acephate	*Mysidopsis bahia*	FT[a]	6.2-7.4	(86-103)	8.0-8.4
	Penaeus duorarum	FT	6.2-7.4	(86-103)	8.1-8.4
	Cyprinodon variegatus	FT	2.7-7.2	(37-99)	6.9-8.3
	Lagodon rhomboides	FT	4.0-6.2	(56-86)	7.6-8.3
Aldicarb	*M. bahia*	S[b]	6.8-7.4	(92-100)	-
	M. bahia	FT	6.4-6.8	(89-94)	8.1-8.4
	P. duorarum	FT	4.6-6.7	(64-93)	8.3
	Penaeus stylirostris	S	5.3-6.3	(72-85)	-
	C. variegatus	S	5.1-7.1	(69-96)	-
	C. variegatus	FT	5.2-6.6	(80-100)	8.3-8.4
	L. rhomboides	FT	4.6-7.0	(66-97)	8.0-8.2
	Leiostomus xanthurus	S	2.4-4.5	(32-61)	-
Carbophenothion	*M. bahia*	S	6.7-7.2	(91-97)	-
	M. bahia	FT	5.2-7.7	(72-101)	8.1-8.2
	P. stylirostris	S	-	-	-
	C. variegatus	S	6.0-7.4	(81-100)	-
	C. variegatus	FT	5.6-7.1	(80-101)	8.0-8.3
	L. rhomboides	FT	3.4-8.1	(47-112)	8.1-8.2
	L. xanthurus	S	3.4-5.8	(46-78)	-
DEF	*M. bahia*	S	6.1-8.6	(82-116)	-
	P. stylirostris	S	5.1-5.9	(69-80)	-
	C. variegatus	S	6.3-7.4	(85-100)	-
	L. xanthurus	S	3.3-4.4	(45-59)	-
EPN	*M. bahia*	S	6.1-8.6	(82-116)	-
	P. stylirostris	S	5.1-5.9	(69-80)	-
	C. variegatus	S	6.3-7.4	(85-100)	-
	L. xanthurus	S	3.3-4.4	(45-59)	-

---

[a] FT = flow-through.

[b] S = static.

Appendix C-8. (Continued)

Ethoprop	*M. bahia*	S	6.1-7.1	(82-96)	-
	M. bahia	FT[a]	3.8-7.0	(53-97)	8.1
	P. duorarum	FT	5.6-7.8	(77-108)	8.0-8.2
	P. stylirostris	S[b]	-	-	-
	C. variegatus	S	5.2-6.7	(70-91)	-
	C. variegatus	FT	5.2-6.4	(82-98)	8.1-8.2
	L. xanthurus	S	2.6-4.7	(35-64)	-
	L. rhomboides	FT	4.4-7.0	(61-97)	8.1
Methyl parathion	*M. bahia*	S	4.3-5.5	(58-74)	-
	P. stylirostris	S	5.6-6.3	(76-85)	-
	Acartia tonsa	S	7.0-7.6	(93-101)	8.1-8.2
	C. variegatus	S	4.6-5.7	(62-77)	-
	L. xanthurus	S	3.2-4.5	(43-61)	-
	L. xanthurus	FT	5.8-7.8	(78-101)	7.8-8.0
Phorate	*M. bahia*	S	2.0-3.8	(32-51)	-
	P. stylirostris	S	4.9-6.3	(66-85)	-
	C. variegatus	S	5.6-6.6	(76-89)	-
	L. xanthurus	S	4.4-4.9	(59-68)	-

---

[a] FT = flow-through.

[b] S = static.

Appendix C-9. Test concentrations and mortality of pinfish (*Lagodon rhomboides*) exposed to acephate in flowing, natural seawater of 28-30 °/oo salinity and 22±1°C. Twenty animals were tested in each treatment.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
65,000	6,200	0	0	0	0
110,000	39,000	0	0	0	0
180,000	49,000	0	0	0	0
300,000	21,000	0	0	5	5
500,000	120,000	0	50	85	95

[a] Hours.

[b] Nondetectable (<400 µg/ℓ).

Appendix C-10. Test concentrations and percentage of sheepshead minnows (*Cyprinodon variegatus*) exhibiting complete loss of equilibrium and lack of escape response after exposure to acephate in flowing, natural seawater. Salinity was 30 $^{0}/oo$ and temperature $22\pm1^{0}C$. Twenty animals were tested in each treatment.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
270,000	100,000	0	0	0	0
450,000	190,000	0	0	0	0
750,000	400,000	0	0	0	0
1,200,000	721,000	0	0	0	0[c]
2,100,000	1,100,000	0	0	100	100

[a] Hours.
[b] Nondetectable (<400 µg/ℓ).
[c] Fish were jittery and lay on the bottom.

Appendix D-1a. Number of normal eastern oyster (*Crassostrea virginica*) embryos per milliliter counted following 48 h of exposure to aldicarb in static, unaerated seawater. If all embryos in the initial inoculum had developed, the expected count would have been 739 embryos per milliliter. Salinity was 20 ‰ and temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Number of normal embryos				
	Rep[a] A	Rep B	Rep C	Mean	SD[b]
Control	611	563	592	588	±24
1,000	565	490	552	535	±40
3,200	419	490	501	470	±44
5,600	368	307	373	349	±36
10,000	143	184	212	179	±34
32,000	208	135	164	169	±36

[a] Replicate.
[b] Standard deviation.

Appendix D-1b. Toxicity of aldicarb to embryos of eastern oysters (Crassostrea virginica) exposed for 48 h in static, unaerated seawater. The criterion of effect was the reduction of the number of normal embryos in test concentrations as compared to the number of normal control embryos. Salinity was 20 $^{0}/oo$ and temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Percentage reduction[a]
Control	-
1,000	9
3,200	20
5,600	41
10,000	70
32,000	71

---

[a] Percentage reduction $= \dfrac{\text{Number of normal 48-h control embryos minus the number of normal 48-h embryos in each test concentration}}{\text{Number of normal 48-h control embryos}} \times 100$

Appendix D-2. Test concentrations and mortality of mysid shrimp (_Mysidopsis bahia_) exposed to aldicarb in static acute toxicity tests using natural seawater adjusted to 20 $^{o}/oo$; temperature $26 \pm 2^{o}C$. Twenty animals were tested in each treatment.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	5	15
Carrier control	0	0	0	0
10.0	5	30	45	45
13.5	5	5	30	55
18	50	60	60	60
24	60	70	80	80
32	40	60	75	95
42	80	95	100	100

[a] Hours.

Appendix D-3. Test concentrations and mortality of postlarval shrimp (*Penaeus stylirostris*) exposed to aldicarb in static acute toxicity tests. Natural seawater was adjusted to 20 ‰; temperature 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	3	22	30	35
Carrier control	20	30	43	48
5.6	0	10	10	10
10	0	5	5	5
18	7	20	30	38
32	15	33	37	43
56	34	44	50	58
100	38	58	83	85
180	50	100	100	100

[a] Hours.

Appendix D-4. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to aldicarb in flowing natural seawater. Salinity was 28 $^{0}/oo$ and temperature, 28±1°C. Twenty animals were tested in each treatment.

Measured concentration (μg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
12.5	0	0	0	0
29.5	0	0	0	0
46.5	10	25	25	25
68.5	40	85	85	85
115	100	100	100	100

[a] Hours.

Appendix D-5. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to aldicarb in static acute toxicity tests using ten animals per treatment. Natural seawater was adjusted to 20 $^{0}/oo$; temperature 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	10	10	10
100	0	10	20	20
320	60	60	60	60
560	100	100	100	100
1,000	100	100	100	100

[a] Hours.

Appendix D-6. Test concentration and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to aldicarb in a flowing seawater test. Shrimp size averaged 5 mm, total length; twenty animals were tested in each treatment. Test temperature was 22±1°C; salinity 28 °/oo.

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
6	2.8	0	0	0	0
11	5.4	0	0	0	0
18	9.7	0	0	0	15
30	14.0	5	5	20	40
50	25.0	0	0	25	80

[a] Hours.
[b] ND = Nondetectable (<0.2 μg/ℓ).

Appendix D-7. Test concentration and mortality of pink shrimp (*Penaeus duorarum*) exposed to aldicarb in a flowing seawater test. Shrimp size ranged 19 to 31 mm (rostrum to telson length). Test temperature was 22±1°C; salinity ranged from 28 to 30 ‰.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
13	6.8	0	0	5	5
22	8.6	15	35	45	45
36	16	35	70	70	75
60	23	40	75	75	80
100	41	90	100	100	100

[a] Hours.

[b] ND = Nondetectable (<0.2 µg/ℓ).

Appendix D-8. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to aldicarb in a flowing seawater test. Fish size ranged 10 to 15 mm, standard length. Test temperature was 28±1°C; salinity 28 $^{o}/oo$.

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
33	12	0	0	0	0
55	29	0	0	0	0
91	46	10	25	25	25
151	68	40	85	85	85
252	120	100	100	100	100

[a] Hours.
[b] ND = Nondetectable (<0.2 μg/ℓ).

Appendix D-9. Test concentrations and mortality of pinfish (*Lagodon rhomboides*) exposed to aldicarb in a flowing seawater test. Fish size ranged from 53 to 90 mm, standard length; twenty animals were tested in each treatment. Test temperature averaged 22±1°C; salinity ranged from 29 to 32 °/oo.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
52	26	0	0	0	0
86	40	0	0	0	0
140	54	0	0	0	0
240	80	50	80	85	85
400	160	100	100	100	100

[a] Hours.

[b] ND = Nondetectable (<0.2 µg/ℓ).

Appendix E-1a. Number of normal eastern oyster (Crassostrea virginica) embryos per milliliter counted following 48-h exposure to carbophenothion (Trithion^R) in static, unaerated seawater. If all embryos in the initial inoculum had developed, the expected count would have been 480 embryos per milliliter. Salinity was 20 ^{o}/oo and temperature, 20±2^{o}C.

Nominal concentration (µg/ℓ)	Number of normal embryos					
	Rep[a] A	Rep B	Rep C	Rep D	Mean	SD[b]
Seawater control	454	409	497	383	436	±50
Carrier control	482	464	446	-	464	±18
0.32	344	343	459	-	382	±67
1.0	409	312	378	-	366	±50
3.2	447	426	458	-	444	±13
10	410	458	486	-	451	±38
32	468	490	351	-	436	±75
100	197	263	182	-	214	±43
320	2	0	1	-	1	±1

[a] Replicate

[b] Standard deviation

Appendix E-1b. Toxicity of carbophenothion to embryos of eastern oysters (*Crassostrea virginica*) exposed for 48 h in static, unaerated seawater. The criterion for effect was the number of normal embryos in test concentrations as compared to the number of normal control embryos. Salinity was 20 °/oo and temperature, 20±2°C.

Nominal concentration (μg/ℓ)	Percentage reduction[a]
Control	-
Carrier control	0
0.32	12
1.0	16
3.2	0
10.0	0
32.0	0
100.0	51
320.0	99.8

[a] Percentage reduction

$$\text{Percentage reduction} = \frac{\text{Number of normal 48-h control embryos minus the number of normal 48-h embryos in each test concentration}}{\text{Number of normal 48-h control embryos}} \times 100$$

Appendix E-2. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to carbophenothion in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 ^{o}/oo; temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	5	10	10
Carrier control	0	5	10	15[b]
3.2	0	0	5	10
5.6	5	15	35	35
10.0	0	10	10	50
18.0	0	35	45	65
32.0	20	80	80	85

[a] Hours.
[b] Excessive control mortality (>10%).

Appendix E-3. Test concentrations and mortality of postlarval penaeid shrimp (Penaeus stylirostris) exposed to carbophenothion in static acute toxicity test using 20 animals per concentration. Natural seawater was adjusted to 20 $^{0}/oo$; temperataure, 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	5	23	33	38[b]
Carrier control	18	35	43	48[b]
0.32	10	20	20	20
0.56	10	10	10	20
1.0	25	38	40	43
1.8	20	28	40	45
3.2	28	48	55	68
5.6	33	55	68	78
10.0	55	85	95	95

[a] Hours.

[b] Excessive control mortality (>10%).

Appendix E-4. Test concencentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to carbophenothion in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 $^0/oo$; temperature 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
10	0	0	0	10
18	5	20	50	55
32	10	85	85	90
56	100	100	100	100
100	100	100	100	100

[a] Hours.

Appendix E-5. Test concencentrations and mortality of spot (Leiostomus xanthurus) exposed to carbophenothion in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 $^{o}/oo$; temperature $25\pm1^{o}C$.

Nominal concentration (μg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
320	0	0	0	10
560	0	30	60	60
1,000	20	70	100	100
1,800	30	90	100	100
3,200	10	100	100	100

[a] Hours.

Appendix E-6. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to carbophenothion in a flowing seawater test. Shrimp averaged 5 mm, total length. Test temperature was 22±1°C; salinity 30 ‰.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
1.7	1.8	0	0	5	20
2.8	2.7	0	0	0	25
4.7	3.6	0	10	15	70
7.8	6.0	0	20	90	100
13.0	15.0	5	45	100	100

[a] Hours.
[b] ND = Nondetectable (<0.08 µg/ℓ).

Appendix E-7. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to carbophenothion in a flowing seawater test. Shrimp size averaged 88 mm rostrum to telson length. Test temperature averaged 25.5°C (25 to 26°C); salinity averaged 24.3 °/oo (19.0 - 29 °/oo).

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	5	5	10
Carrier control	ND	0	0	0	0
0.056	0.12	0	10	10	10
0.1	0.14	0	15	15	20
0.18	0.29	0	10	10	20
0.32	0.48	0	10	15	45
0.56	0.79	0	10	85	90

[a] Hours.
[b] ND = Nondetectable (<0.02 μg/ℓ).

Appendix E-8. Test concentration and mortality of grass shrimp (Palaemonetes pugio) larvae (1- to 7-day-old) exposed to carbophenothion in a flowing seawater test. Test temperature averaged 25.5°C (25 to 26°C); salinity 25 ‰. Forty larvae were placed in each treatment.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	2.5	5.0	5.0	7.5
Carrier control	ND	0	2.5	7.5	12.5
1.15	1.0	2.5	5.0	5.0	12.5
1.55	1.3	15.0	15.0	15.0	25.5
2.1	1.8	17.5	17.5	22.5	27.5
2.8	2.1	12.5	12.5	45.0	65.0
3.7	2.6	15.0	22.5	65.0	82.5

[a] Hours.

[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix E-9. Test concentrations and mortality of grass shrimp (*Palaemonetes pugio*) exposed to carbophenothion in a flowing seawater test. Shrimp size averaged 15.4 mm, rostrum-to-telson length; twenty animals were tested in each treatment. Test temperature averaged 29.1°C (29.0 to 29.5°C); salinity 27.4 °/oo (25.5 to 29 °/oo).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
0.75	0.78	0	0	0	0
1.35	1.30	0	0	0	0
2.4	1.8	0	0	0	5
4.2	3.5	0	30	45	75
7.5	5.5	15	100	100	100

[a] Hours.

[b] ND = nondetectable (<0.02 µg/ℓ).

Appendix E-10. Test concentrations and mortality of wild-stock grass shrimp (Palaemonetes pugio) exposed to carbophenothion in a flowing seawater test. Shrimp size averaged 16.6 mm, rostrum to telson length; twenty animals were tested in each treatment. Test temperature averaged 29.1°C (29.0-29.5°C); salinity averaged 27.4 °/oo (25.5 to 29.0 °/oo).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
0.75	0.78	0	0	0	0
1.35	1.3	0	0	0	0
2.4	1.8	0	0	0	0
4.2	3.5	0	5	25	30
7.5	5.5	0	40	85	100

[a] Hours.

[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix E-11. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to carbophenothion in a flowing seawater test. Fish size ranged from 10 to 15 mm, standard length. Test temperature was 28±1°C; salinity 28 ‰.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
1.7	0.5	0	0	0	0
2.8	0.6	0	0	0	0
4.7	1.0	0	0	15	15
7.8	1.6	0	5	5	10
13	4.0	10	10	40	70

[a] Hours.

[b] ND = Nondetectable (<0.08 µg/ℓ).

Appendix E-12. Test concentrations and mortality of Atlantic silversides (*Menidia menidia*) exposed to carbophenothion in a flowing seawater test. Fish size averaged 11 mm, standard length; twenty animals were tested in each treatment. Test temperature averaged 24.9°C (23 to 26°C); salinity 25.5 ‰ (24 to 26 ‰).

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
1.8	2.2	10	15	20	20
3.2	4.9	5	5	10	25
5.6	6.9	20	20	20	35
7.5	7.5	20	35	35	55
13.5	12.9	35	65	75	75

[a] Hours.

[b] ND = Nondetectable (<0.02 μg/ℓ).

Appendix E-13. Test concentrations and mortality of spot (Leiostomus xanthurus) exposed to carbophenothion in a flowing seawater test. Fish size averaged 30.7 mm, standard length; twenty animals were tested in each treatment. Test temperature averaged 26.2°C (26 to 26.5°C); salinity 25.5 °/oo (19 to 28 °/oo).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
42	-	0	0	0	0
75	-	0	0	0	0
135	28	0	0	0	0
240	35	0	0	5	5
420	206	0	0	35	35

[a] Hours.
[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix E-14. Test concentrations and mortality of pinfish (*Lagodon rhomboides*) exposed to carbophenothion in a flowing seawater test. Fish size ranged from 53 to 90 mm, standard length. Twenty animals were tested in each treatment.

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
13	4.9	0	0	0	0
22	5.5	0	0	0	0
36	5.2	0	0	0	30
60	6.2	0	0	5	70
100	34	0	0	100	100

[a] Hours.
[b] ND = Nondetectable (<0.08 μg/ℓ).

Appendix E-15. Mean salinity and dissolved oxygen during a 249-day partial life-cycle toxicity test of carbophenothion with grass shrimp (*Palaemonetes pugio*). The test tank temperature averaged 25.5°C (range 22.4 - 28.3°C), whereas the larval tray temperature averaged 25.0°C (range 22.2 - 30°C) for the entire exposure.

Test day	Salinity (°/oo)		Dissolved oxygen (mg/ℓ) for each treatment							
	Mean	Range	Control	Carrier control	0.06	0.22	0.36	0.69	1.31	2.94
1-8	22.9	21-26	6.7	5.5	5.9	6.2	5.5	5.8	5.9	5.5
9-39	25.7	21-30	6.3	5.0	4.8	5.3	5.1	5.3	5.1	5.4
40-69	28.7	27-30	5.7	3.0	3.4	3.2	3.6	3.5	3.0	4.2
70-100	24.6	21-30	6.1	3.2	4.1	4.4	4.2	4.4	4.4	4.9
101-131	22.7	18-27	6.2	3.0	3.7	4.4	4.1	4.2	4.1	5.2
132-159	21.2	18-27	6.2	4.1	2.5	3.0	3.3	4.2	4.0	4.6
160-190	17.5	10-24	6.0	2.2	3.3	3.8	2.7	4.1	3.6	5.1
191-220	23.5	20-26	6.2	5.5	5.3	5.6	4.9	4.3	5.3	6.7
221-249	23.7	22-25	7.0	6.4	6.3	6.4	6.4	6.4	6.2	6.4

Appendix F-1a. Number of normal eastern oyster (Crassostrea virginica) embryos per milliliter counted following 48 h of exposure to DEF in static, unaerated seawater. If all embryos in the initial inoculum had developed, the expected count would have been 1,059 embryos per milliliter. Salinity was 20 ‰ and temperature 25±1°C.

Nominal concentration (µg/ℓ)	Number of normal embryos				
	Rep[a] A	Rep B	Rep C	Mean	SD[b]
Control	714	827	793	778	±57
Carrier control	758	812	809	793	±30
100	786	753	731	756	±27
180	498	426	384	436	±57
320	187	231	212	210	±22
560	0	0	0	0	0
1,000	0	0	0	0	0

[a] Replicate.
[b] Standard deviation.

Appendix F-1b. Toxicity of DEF to embryos of eastern oysters (*Crassostrea virginica*) exposed for 48 h in static, unaerated seawater. The criterion for effect was the reduction of the number of normal embryos in test concentrations as compared to the number of normal control embryos. Salinity was 20 $^{o}/oo$ and temperature, 25±1°C.

Nominal concentration ($\mu g/\ell$)	Percentage reduction[a]
Control	-
Carrier control	-
100	3
180	44
320	73
560	100
1,000	100

---

[a] $$\text{Percentage reduction} = \frac{\text{Number of normal 48-h control embryos minus the number of normal 48-h embryos in each test concentration}}{\text{Number of normal 48-h control embryos}} \times 100$$

Appendix F-2. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to DEF in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 $^{o}/oo$; temperature was 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	5	5	5	5
5.6	5	10	30	45
10.0	0	45	90	95
18.0	5	80	95	100
32.0	10	95	100	100
56.0	15	100	100	100

[a] Hours.

Appendix F-3. Test concentrations and mortality of postlarval shrimp (*Penaeus stylirostris*) exposed to DEF in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 $^{o}/oo$; temperature 26±2°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	8	18	25	33[b]
Carrier control	10	13	23	28[b]
3.2	10	10	10	20
5.6	3	8	20	28
10.0	5	13	20	23
18.0	18	43	60	63
32.0	25	48	60	68
56.0	50	55	100	100

[a] Hours.
[b] Excessive control mortality (>10%).

Appendix F-4. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to DEF in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 $^{o}/oo$; temperature $25 \pm 1^{o}C$.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
240	0	0	0	0
320	0	0	0	5
420	0	0	10	25
560	0	40	90	100
750	35	95	100	100

[a] Hours.

Appendix F-5. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to DEF in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 °/oo; temperature 26±1°C.

Nominal concentration (μg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Control carrier	3	3	3	3[b]
56	0	0	7	7
75	0	0	0	0
100	0	0	7	13
135	0	0	0	10
180	0	0	43	83
320	10	90	100	100
560	100	100	100	100

[a] Hours.
[b] Excessive control mortality (>10%).

Appendix F-6. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to DEF in a flowing seawater test. Early juveniles (24 h; total length ≈ 1 mm) were used. Test temperature averaged 25°C (24-26°C); test salinity 16 ‰ (15-17 ‰).

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	5	10	10
Carrier control	ND	0	0	0	0
1.0	0.58	0	5	5	5
2.0	1.6	0	0	0	0
4.0	3.7	0	10	15	15
8.0	5.6	10	25	45	90

[a] Hours.

[b] Nondetectable (<0.02 μg/ℓ).

Appendix F-7. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to DEF in a flowing seawater test. Shrimp size averaged 84 mm, rostrum to telson length; twenty animals were tested in each treatment. Test temperature averaged 25.6°C (25 to 26°C); salinity 20 $^{o}/oo$ (15-26 $^{o}/oo$).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	5	5
4.2	4.5	0	0	5	5
7.5	6.9	20	20	25	25
13.5	9.7	5	5	15	45
24.0	16.0	35	50	60	70
42.0	29.0	50	75	85	90

[a] Hours.
[b] Nondetectable (<0.02 µg/ℓ).

Appendix F-8. Test concentration and mortality of wild-stock grass shrimp (*Palaemonetes pugio*) exposed to DEF in flowing seawater tests. Shrimp size averaged 16.6 mm, rostrum to telson length; twenty animals were used in each treatment. Test temperature averaged 28.1°C (27.5-29.0°C); salinity 21.6 °/oo (16.0 to 28 °/oo).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	5	5
Carrier control	ND	0	0	0	0
13.5	16	0	10	20	20
24	20	0	20	40	40
42	29	5	80	100	100
75	80	0	70	100	100
135	160	40	100	100	100

[a] Hours.

[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix F-9. Test concentration and mortality of pinfish (*Lagodon rhomboides*) exposed to DEF in flowing seawater tests. Fish size averaged 24 mm, standard length; twenty animals were used in each treatment. Test temperature averaged 24.9°C (24.5 to 25.5°C); salinity 17.9 ^{0}/oo (12 to 23 ^{0}/oo).

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
75	47	0	0	0	0
135	69	0	0	0	0
180	189	0	0	15	30
240	240	0	0	0	40
320	343	0	0	10	60

[a] Hours.

[b] ND = Nondetectable (<0.02 μg/ℓ).

Appendix F-10. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to DEF in flowing seawater tests. Fish averaged 17 mm, standard length; twenty animals were used in each treatment. Test temperature averaged 25.2°C (25 to 26°C); salinity 18.2 ‰ (15.5 to 23.5 ‰).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
24	23	0	0	0	0
42	59	0	0	0	5
75	90	0	0	0	10
135	162	0	0	25	85

[a] Hours.

[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix F-11. Test concentrations and mortality of sheepshead minnow (*Cyprinodon variegatus*) exposed to DEF in a flowing seawater test. Fish size averaged 8.7 mm, standard length; twenty animals were used in each treatment. Test temperature averaged 15.4°C (14.5 to 16.5°C); salinity 17.9 ‰ (12 to 21 ‰).

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
135	47	0	0	0	0
320	270	0	0	0	0
420	330	0	0	0	0
750	440	0	0	0	0

[a] Hours.

[b] ND = Nondetectable (<0.02 μg/ℓ).

Appendix G-1a. Number of normal eastern oyster (*Crassostrea virginica*) embryos per milliliter counted following 48 h of exposure to EPN in static, unaerated seawater. If all embryos in the initial inoculum had developed, the expected count would have been 739 embryos per milliliter. Salinity was 20 $^{o}/oo$ and temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Number of normal embryos				
	Rep[a] A	Rep B	Rep C	Mean	SD[b]
Control	611	563	592	588	±24
Carrier control	548	587	579	571	±20
320	528	606	549	561	±40
560	517	493	558	522	±32
1,000	490	529	509	509	±19
3,200	190	106	150	148	±42
5,600	184	98	137	139	±43

[a] Replicate.
[b] Standard deviation.

Appendix G-1b. Toxicity of EPN to embryos of eastern oysters (*Crassostrea virginica*) exposed for 48 h in static, unaerated seawater. Criterion of effect was reduction of the number of normal embryos. Salinity was 20 $^{o}/oo$ and temperature $25 \pm 1^{o}C$.

Nominal concentration (µg/ℓ)	Percentage reduction[a]
Control	-
Carrier control	3
320	4
560	11
1,000	13
3,200	75
5,600	76

$$\text{[a]Percentage reduction} = \frac{\text{Number of normal 48-h control embryos minus the number of normal 48-h embryos in each test concentration}}{\text{Number of normal 48-h control embryos}} \times 100$$

Appendix G-2. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to EPN in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 ^{0}/oo; temperature, 25±1^{0}C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	5	0	10
Carrier control	0	0	0	0
3.2	0	0	10	10
5.6	0	5	5	15
10.0	5	5	10	15
18.0	0	30	75	95
32.0	50	80	100	100

[a] Hours.

Appendix G-3. Test concentrations and mortality of postlarval penaeid shrimp (*Penaeus stylirostris*) exposed to EPN in static acute toxicity tests using 40 or 60 animals per concentration. Natural seawater was adjusted to 20 ‰; temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	7	10	23	28
Carrier control	7	15	22	25
1.0	0	10	15	23
1.8	23	33	40	47
3.2	17	28	33	40
5.6	20	43	57	60
10.0	38	72	83	93
18.0	67	90	92	98

[a] Hours.

Appendix G-4. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to EPN in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 ^{o}/oo; temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
32	0	0	0	0
56	0	0	15	15
100	0	5	10	15
180	30	45	50	55
320	75	85	100	100

[a] Hours.

Appendix G-5. Test concentrations and mortality of spot (Leiostomus xanthurus) exposed to EPN in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 $^{0}/oo$; temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	10	10	10
Carrier control	0	0	20	20
24	0	0	0	0
32	0	0	0	20
42	0	40	70	90
56	0	80	100	100
75	0	20	90	90

[a] Hours.

Appendix G-6. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to EPN in a flowing seawater test. Mean total length of adult mysids used was 7.5 mm; twenty animals were tested in each treatment. Test temperature averaged 23°C (22 to 24°C); salinity 24 ‰ (22 to 26 ‰).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	5
1.0	1.1	0	0	0	5
2.0	1.7	0	0	5	15
4.0	3.8	5	10	20	60
8.0	8.6	15	65	85	100

[a] Hours.
[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix G-7. Test concentrations and mortality of pink shrimp (Penaeus duorarum) exposed to EPN in a flowing seawater test. Shrimp size averaged 69 mm, rostrum-to-telson length; twenty animals were tested in each treatment. Test temperature averaged 25°C (24 to 26°C); test salinity 24 °/oo (22 to 26 °/oo).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	5
0.032	ND	0	10	20	20
0.1	0.042	0	5	10	10
0.32	0.42	5	20	35	45
1.0	1.3	95	100	100	100
3.2	-	90	100	100	100

[a] Hours.
[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix G-8. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to EPN in a flowing seawater test. Fish size averaged 14 mm standard length; twenty animals were tested in each treatment. Test temperature averaged 24.6°C (24.0 to 25.3°C); test salinity 18.2 ‰ (12.5 to 24.5 ‰).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
75	58	10	25	30	35
140	76	15	25	40	45
240	150	65	90	90	90
420	240	80	100	100	100
750	-	100	100	100	100

[a] Hours.

[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix G-9. Test concentrations and mortality of spot (Leiostomus xanthurus) exposed to EPN in a flowing seawater test. Fish size averaged 51 mm, standard length; twenty animals were tested in each treatment. Test temperature averaged 24.0°C (23 to 25°C); test salinity 23 °/oo (20 to 26 °/oo).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
3.2	3.2	0	0	0	0
5.6	5.9	0	0	0	5
10	12	0	0	0	15
18	28	0	15	20	45
32	42	0	0	100	100

[a] Hours.
[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix G-10. Test concentrations and mortality of pinfish (Lagodon rhomboides) exposed to EPN in a flowing seawater test. Fish size averaged 64 mm, standard length; twenty animals were used in each treatment. Test temperature averaged 25°C (24 to 26°C); test salinity 28.8 ‰ (27 to 32 ‰).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
3.2	3.0	0	0	0	0
5.6	5.0	0	0	0	0
10	6.7	0	0	0	0
18	12	0	5	10	15
32	26	40	80	90	90

[a] Hours.
[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix G-11. Effect of EPN on the swimming performance of *Cyprinodon variegatus*

by

Geraldine M. Cripe

Measurements of swimming ability of toxicant-exposed fish may indicate changes in physiological conditions at sublethal levels. Pulpwood fiber (MacLeod and Smith, 1966), the herbicide, diquat (Dodson and Mayfield, 1979) and the organophosphate, fenitrothion (Peterson, 1974) have adversely affected laboratory-measured swimming performance of fish at sublethal levels. This study examined the effects of EPN on the swimming ability of *Cyprinodon variegatus*.

Swimming performance of adult *Cyprinodon variegatus* (averaging 3.7 cm, standard length) was measured using a stamina tunnel of 2-inch inside diameter clear plexiglass tubing. The fish were chronically exposed for 256 days in the TEG control and in EPN at measured concentrations 0.25, 0.5, 0.88, 2.2, and 4.1 µg/ℓ. Nine to ten fish from each treatment were tested individually to determine their critical swimming speed. This measurement was made using step increases in velocity at 2-minute intervals and a calculation devised by Brett (Brett, 1964):

CSS = LRV + VI (TE/TT)

where

CSS = critical swimming speed (cm/sec)

LRV = last recorded velocity which was maintained for the prescribed time period (cm/sec)

TE = time endured at the final velocity (sec)

TT = total time increment (sec)

VI = velocity increment between final velocity and the preceding velocity (cm/sec)

The critical swimming speeds for the fish tested are shown in Table a. Only CSS measurements from animals exposed to 2.2 and 4.1 μg EPN/ℓ were significantly different from controls, therefore, the threshold level of effect on swimming performance lies between 2.2 and 0.38 μg EPN/ℓ.

From these tests, one could speculate that a fish in the field exposed to these compounds might also show a reduced swimming performance. This loss may affect its ability to remain in favorable environmental conditions (light, oxygen levels, temperature and food supply) against the influence of ebb and flood tides. It also may be important in terms of spawning behavior such as territorial defense and in predator avoidance of prey capture.

Literature Cited

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Canada 21:1184-1226.

Dodson, J.J. and C.I. Mayfield. 1979. Modification of the rheotropic response of rainbow trout (*Salmo gairdneri*) by sublethal doses of the aquatic herbicides diquat and simazine. Environ. Pollut. 18:147-157.

MacLeod, J.C. and L.L. Smith, Jr. 1966. Effect of pulpwood fiber on oxygen consumption and swimming endurance of the fathead minnow, *Pimephales promelas*. Trans. Amer. Fish. Soc. 95: 71-84.

Peterson, R.H. 1974. Influence of fenitrothion on swimming velocities of brood trout (*Salvelinus fontinalis*). J. Fish. Res. Bd. Canada 31:1757-1762.

Table a. Critical swimming speed (cm/sec) of individual sheepshead minnows continuously exposed to measured concentrations of EPN for 256 days.

Exposure Concentration (μg/ℓ)	Fish Number											
	1	2	3	4	5	6	7	8	9	10	$\bar{X}$	SD
ND[a]	39.3	46.4	52.0	52.0	25.6	52.0	42.4	52.0	52.0	52.0	46.6	8.7
0.25	19.4	52.0	46.3	51.4	52.0	52.0	41.9	47.7	21.1	52.0	43.6	12.7
0.5	52.0	42.3	51.6	52.0	33.9	52.0	27.2	15.9	52.0	16.0	39.5	15.2
0.88	41.3	52.0	44.9	33.0	52.0	52.0	52.0	35.4	37.2	3.0	40.3	15.1
2.2	0.3	17.2	33.2	14.6	45.8	28.3	7.4	39.4	26.0	52.0	26.4[b]	16.7
4.1	36.8	0.3	52.0	18.3	9.6	14.7	17.8	4.3	40.3		21.6[b]	17.6

[a] Control-with-carrier, ND = nondetectable (<0.05 μg/ℓ).
[b] Significantly different from control ($\alpha = 0.05$).

Appendix H-1a. Number of normal eastern oyster (Crassostrea virginica) embryos per milliliter counted following 48 h of exposure to ethoprop in static, unaerated seawater. If all embryos in the initial inoculum had developed, the expected count would have been 1,059 embryos per milliliter. Salinity was 20 ‰ and temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Number of normal embryos				
	Rep[a] A	Rep B	Rep C	Mean	SD[b]
Control	714	827	793	778	±57
Carrier control	758	821	809	793	±30
3,200	731	773	805	769	±37
5,600	724	653	682	686	±35
10,000	395	438	335	402	±32
32,000	217	280	178	225	±51
56,000	66	46	34	48	±16

[a] Replicate.

[b] Standard deviation.

Appendix H-1b. Toxicity of ethoprop to embryos of eastern oysters (*Crassostrea virginica*) exposed for 48 h in static, unaerated seawater. Criterion of effect was reduction of the number of normal embryos in test concentrations as compared to the number of normal control embryos. Salinity was 20 $^{0}/oo$ and temperature 25±1°C.

Nominal concentration (µg/ℓ)	Percentage reduction[a]
Control	-
Carrier control	-
3,200	1
5,600	12
10,000	48
32,000	71
56,000	94

$$^{a}\text{Percentage reduction} = \frac{\text{Number of normal 48-h control embryos minus number of normal 48-h embryos in each test concentration}}{\text{Number of normal 48-h control embryos}} \times 100$$

Appendix H-2. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to ethoprop in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 $^0/oo$; temperature 25±1^{0}C.

Nominal concentration (μg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	5	5	5
10	0	5	10	10
18	0	0	5	15
32	0	15	60	80
56	45	95	100	100
100	95	100	100	100

[a] Hours.

Appendix H-3. Test concentrations and mortality of postlarval penaeid shrimp (*Penaeus stylirostris*) exposed to ethoprop in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 ^{o}/oo; temperature 25±2^{o}C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	3	3	5	8
Carrier control	3	13	20	23[b]
1.8	0	0	5	15
3.2	0	0	20	25
5.6	0	0	25	35
10	8	45	60	90
18	95	100	100	100
32	100	100	100	100

---

[a] Hours.

[b] Excessive control mortality (>10%).

Appendix H-4. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to ethoprop in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 ^{0}/oo; temperature 25 ± 1^{0}C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
100	0	0	0	0
180	0	0	0	0
320	0	5	15	15
560	5	30	35	35
1,000	30	55	60	65

[a] Hours.

Appendix H-5. Test concentrations and mortality of spot (Leiostomus xanthurus) exposed to ethoprop in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 $^{0}/oo$; temperature 25±1^{0}C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	17	17[b]
32	0	0	33	50
56	50	50	83	83
100	83	83	100	100
180	100	100	100	100
320	100	100	100	100

[a] Hours.
[b] Excessive control mortality (>10%).

Appendix H-6. Test concentration and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to ethoprop in a flowing seawater test. Shrimp size averaged 5 mm, total length; twenty animals were tested in each treatment. Test temperature was 22±1°C; salinity 28±1 ‰.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
4	2.2	5	5	5	10
6	4.9	0	5	10	20
11	6.8	5	10	20	35
18	7.7	0	10	25	55
30	14.0	20	60	75	100

[a] Hours.
[b] ND = Nondetectable (<0.1 µg/ℓ).

Appendix H-7. Test concentration and mortality of pink shrimp (*Penaeus duorarum*) exposed to ethoprop in a flowing seawater test. Shrimp size ranged from 36-64 mm (rostrum to telson length); twenty animals were used in each treatment. Test temperature was 22±1°C; salinity 26 to 30 ‰

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
4	2	0	0	0	0
6	2.6	0	0	0	0
11	5.6	0	0	0	5
18	8.2	0	0	15	20
30	19	0	0	45	75

[a] Hours.

[b] ND = Nondetectable (<0.5 µg/ℓ).

Appendix H-8. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to ethoprop in a flowing seawater test. Fish size ranged from 10 to 15 mm, standard length; twenty animals were used in each treatment. Test temperature was 30±1°C; salinity 28±1 °/oo.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	5	10	10
Carrier control	ND	0	20	20	20[c]
130	140	0	15	30	40
216	220	0	15	30	50
360	280	0	35	70	90
600	600	20	80	95	95
1,000	1,100	25	90	100	100

[a] Hours.
[b] ND = Nondetectable (<0.5 µg/ℓ).
[c] Unacceptable control mortality (>10%).

Appendix H-9. Test concentrations and mortality of pinfish (Lagodon rhomboides) exposed to ethoprop in a flowing seawater test. Fish size ranged from 53 to 90 mm, standard length; twenty animals were tested in each treatment. Test temperature was 22±1°C; salinity 28 to 30 °/oo.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
4	2.1	0	0	0	0
6	4.9	0	5	5	5
11	6.7	0	15	25	30
18	7.7	15	65	70	75
30	14	20	100	100	100

[a] Hours.

[b] ND = Nondetectable; (<0.5 µg/ℓ).

Appendix I-1a. Number of normal eastern oyster (*Crassostrea virginica*) embryos per milliliter counted following 48 h of exposure to methyl parathion in static, unaerated seawater. If all embryos in the initial inoculum had developed, the expected count would have been 1,059 embryos per milliliter. Salinity was 20 ‰ and temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Number of normal embryos				
	Rep[a] A	Rep B	Rep C	Mean	SD[b]
Control	714	827	793	778	±57
3,200	598	523	609	576	±46
5,600	518	552	523	531	±18
10,000	335	312	271	306	±32
32,000	301	264	298	287	±20
56,000	198	253	219	223	±27

[a] Replicate.
[b] Standard deviation.

Appendix I-1b. Toxicity of methyl parathion to embryos of eastern oysters (Crassostrea virginica) exposed for 48 h in static, unaerated seawater. Criterion of effect was reduction of the number of normal embryos in test concentrations as compared to the number of normal control embryos. Salinity was 20 ^{o}/oo and temperature 25±1°C.

Nominal concentration (μg/ℓ)	Percentage reduction[a]
Control	-
3,200	26
5,600	31
10,000	61
32,000	63
56,000	71

$$\text{[a]Percentage reduction} = \frac{\text{Number of normal 48-h control embryos minus the number of normal 48-h embryos in each test concentration}}{\text{Number of normal 48-h control embryos}} \times 100$$

Appendix I-2. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to methyl parathion in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 ^{o}/oo; temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
0.18	0	0	0	0
0.32	0	0	0	0
0.56	10	10	20	20
1.0	5	20	40	45
1.8	70	90	90	90

[a] Hours.

Appendix I-3. Test concentrations and mortality of postlarval penaeid shrimp (Penaeus stylirostris) exposed to methyl parathion in static acute toxicity test using 20 animals per concentration. Natural seawater was adjusted to 20 $^{o}/oo$; temperature, 25±2°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	7	15	32	40
Carrier control	7	18	23	33
0.32	8	25	33	33
0.56	10	22	30	42
1.0	18	40	53	57
1.8	38	55	65	70
3.2	84	90	93	98
5.6	100	100	100	100

[a] Hours.

Appendix I-4. Test concentrations and mortality of calanoid copepod (*Acartia tonsa*) exposed for 96 h to methyl parathion in static, unaerated seawater. Salinity was 22 ⁰/oo and temperature, 22±1°C.

Nominal concentration (μg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	3	0
Carrier control	0	0	0	3
13	7	10	13	20
22	3	3	10	27
36	3	13	27	43
60	17	37	53	67
100	43	63	80	100

[a] Hours.

Appendix I-5. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to methyl parathion in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 ⁰/oo; temperature 25±1°C.

Nominal concentration (μg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
10,000	0	0	0	0
12,000	5	15	25	35
14,000	85	95	100	100
16,000	70	100	100	100
18,000	100	100	100	100

[a] Hours.

Appendix I-6. Test concentrations and mortality of spot (Leiostomus xanthurus) exposed to methyl parathion in static acute toxicity tests using 20 animals per concentration. Natural seawater was adjusted to 20 ⁰/oo; temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
56	0	0	0	0
100	0	20	50	60
180	0	20	40	70
320	30	70	90	100
560	50	70	80	90

[a] Hours.

Appendix I-7. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to methyl parathion in a flowing seawater test. Early juveniles (<48h; total length <2 mm) were used. Twenty animals were tested in each treatment. Test temperature averaged 19.5°C (18-21°C); test salinity 14 °/oo (12 to 16 °/oo).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Carrier control	ND[b]	0	0	0	0
0.19	0.15	0	0	0	5
0.38	0.41	0	0	15	20
0.75	0.68	0	0	25	25
1.5	1.3	0	15	90	90

[a] Hours.

[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix I-8. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to methyl parathion in flowing seawater tests. Shrimp size averaged 88 mm, rostrum to telson length; twenty animals were tested in each treatment. Test temperature averaged 24.8°C (24.5 to 25.0°C); test salinity 21.6 ‰ (17.0 to 27.0 ‰).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
0.56	0.57	0	0	0	15
0.75	0.79	20	20	20	25
1.35	1.7	80	80	80	80
1.8	1.8	95	95	100	100
2.4	2.3	90	90	95	95

[a] Hours.

[b] ND = Nondetectable (<0.02 µg/ℓ).

Appendix I-9. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to methyl parathion in flowing, natural seawater. Twenty animals were tested in each treatment. Salinity was 10-15 °/oo and temperature 22-23°C.

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
65	25	5	5	10	10
108	56	0	15	40	40
180	77	30	55	65	75
300	130	60	70	75	85
500	250	100	100	100	100

[a] Hours.
[b] ND = Nondetectable; (<0.5 µg/ℓ).

Appendix J-1a. Number of normal eastern oyster (*Crassostrea virginica*) embryos per milliliter counted following 48 h of exposure to phorate in static, unaerated seawater. If all embryos in the initial inoculum had developed, the expected count would have been 739 embryos per milliliter. Salinity was 20 ‰ and temperature 25±1°C.

Nominal concentration (µg/ℓ)	Number of normal embryos				
	Rep[a] A	Rep B	Rep C	Mean	SD[b]
Control	611	563	592	588	±24
Carrier control	548	587	579	574	±20
320	518	504	545	522	±20
560	400	455	378	411	±39
1,000	348	376	410	378	±31
3,200	83	165	130	126	±41
5,600	0	0	0	0	0

[a] Replicate.
[b] Standard deviation.

Appendix J-1b. Toxicity of phorate to embryos of eastern oysters (Crassostrea virginica) exposed for 48 h in static, unaerated seawater. Criterion of effect was reduction of the number of normal embryos in test concentrations as compared to the number of control embryos. Salinity was 20 $^o/oo$ and temperature, 25±1°C.

Nominal concentration (μg/ℓ)	Percentage reduction[a]
Control	-
Carrier control	2
320	11
560	30
1,000	36
3,200	78
5,600	100

[a] $$\text{Percentage reduction} = \frac{\text{Number of normal 48-h control embryos minus the number of normal 48-h embryos in each test concentration}}{\text{Number of normal 48-h control embryos}} \times 100$$

Appendix J-2. Test concentrations and mortality of postlarval penaeid shrimp (Penaeus stylirostris) exposed to phorate in static acute toxicity tests using 20 animals per concentration. Seawater was adjusted to 20 $^{o}/oo$; temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	5	8
Carrier control	10	15	23	28
0.18	8	13	18	18
0.32	20	63	80	85
0.56	58	83	88	90
1.0	100	100	100	100
1.8	95	100	100	100

[a] Hours.

Appendix J-3. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to phorate in static acute toxicity tests using 20 animals per concentration. Seawater was adjusted to 20 ^{0}/oo; temperature, 25±2°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
0.32	5	5	5	5
0.56	10	10	10	30
1.0	5	10	15	20
1.8	5	15	35	45
3.2	20	40	90	95

[a] Hours.

Appendix J-4. Test concentrations and mortality of sheepshead minnows (Cyprinodon variegatus) exposed to phorate in static acute toxicity tests using 20 animals per concentration. Seawater was adjusted to 20 ‰; temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	0
Carrier control	0	0	0	0
2.4	5	5	5	5
3.2	20	40	45	50
4.2	30	40	45	50
5.6	60	70	70	70
7.5	95	95	95	95

[a] Hours.

Appendix J-5. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to phorate in static acute toxicity tests using 20 animals per concentration. Seawater was adjusted to 20 ^{0}/oo; temperature, 25±1°C.

Nominal concentration (µg/ℓ)	Mortality (%)			
	24h[a]	48h	72h	96h
Control	0	0	0	5
Carrier control	10	10	15	15
3.2	0	0	0	0
4.2	0	10	10	10
5.6	45	90	90	90
7.5	95	100	100	100
10.0	100	100	100	100

[a] Hours.

Appendix J-6. Test concentrations and mortality of pink shrimp (*Penaeus duorarum*) exposed to phorate in a flowing seawater test. Shrimp size averaged 88 mm, rostrum-to-telson length; twenty animals were tested in each treatment. Test temperature averaged 25.3°C (25 to 26°C); test salinity 24.9 °/oo (22 to 28.5 °/oo).

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier Control	ND	0	0	0	5
0.056	0.08	5	5	5	10
0.18	0.12	15	30	60	70
0.32	0.26	50	100	100	100
0.56	0.49	85	100	100	100
1.8	1.7	100	100	100	100

[a] Hours.

[b] ND = Nondetectable (<0.02 μg/ℓ).

Appendix J-7. Test concentrations and mortality of mysid shrimp (*Mysidopsis bahia*) exposed to phorate in a flowing seawater test. Twenty early juveniles (<48h; total length ≈ 2 mm) were tested in each treatment. Test temperature averaged 24.7°C (24 to 25°C); test salinity 19.4 ^{o}/oo (16 to 27 ^{o}/oo).

Concentration (μg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	10	10	10
Carrier control	ND	0	5	5	5
0.32	0.31	5	20	30	30
0.56	0.37	0	35	60	75
1.0	0.75	5	80	85	100
1.8	1.5	60	100	100	100
3.2	1.7	95	100	100	100

[a] Hours.

[b] ND = nondetectable (<0.02 μg/ℓ).

Appendix J-8. Test concentrations and mortality of sheepshead minnows (*Cyprinodon variegatus*) exposed to phorate in flowing seawater test. Fish size averaged 6.6 mm, standard length; twenty animals were tested in each treatment. Test temperature averaged 25°C (24 to 25°C); test salinity 16.9 °/oo (13 to 21 °/oo).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
0.14	0.12	0	0	0	0
0.24	0.22	0	0	0	0
0.42	0.50	0	0	0	0
0.75	0.50	0	0	0	5
1.40	1.50	20	20	25	70

[a] Hours.

[b] ND = nondetectable (<0.02 µg/ℓ).

Appendix J-9. Test concentrations and mortality of spot (*Leiostomus xanthurus*) exposed to phorate in flowing seawater test. Fish size averaged 24 mm, standard length; twenty animals were tested in each treatment. Test temperature averaged 24.8°C (24 to 26°C); test salinity 16.9 °/oo (10.0 to 25 °/oo).

Concentration (µg/ℓ)		Mortality (%)			
Nominal	Measured	24h[a]	48h	72h	96h
Control	ND[b]	0	0	0	0
Carrier control	ND	0	0	0	0
0.42	0.39	0	0	0	0
0.75	0.47	0	0	0	0
1.4	1.1	0	0	0	10
2.4	2.8	0	25	25	25
4.2	4.2	0	45	50	55

[a] Hours.

[b] ND = nondetectable (<0.02 µg/ℓ).